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THE DIFFUSION OF CARBON DIOXIDE THROUGH SOILS[1]

F. B. SMITH AND P. E. BROWN

Iowa Agricultural Experiment Station

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The importance of carbon dioxide production in soils has long been recognized, and various methods have been proposed for its determination. The first determinations involved measurements of the concentration of carbon dioxide in the soil air. It was assumed that a high concentration of carbon dioxide was synonymous to a high rate of production. However, it was soon found that although this may be true generally, it is not necessarily true under all conditions. The carbon dioxide produced may accumulate in one soil to a greater extent than in another even though the rate of production is the same in both cases. In other words, the concentration of carbon dioxide in the soil air, or its accumulation in the pore space of the soil, is a function not only of the rate of production but also of the rate of escape. Carbon dioxide produced in the soil may diffuse in any direction from the place where it is produced, it may go into solution, or it may be fixed in the soil.

The evolution of carbon dioxide from small samples of soils in the laboratory was next considered a measure of the rate of production. The desirability of measurements of rate of production in soils *in situ* was early recognized, and certain methods were adapted for that purpose. However, the evolution of carbon dioxide from a small sample of soil, although far from giving an accurate measure of the rate of carbon dioxide production, is perhaps still the most reliable method for its determination.

Lundegardh (3) defined *soil respiration* as the amount of carbon dioxide in grams evolved from 1 sq.m. of soil *in situ* in 1 hour, and the rate of respiration was considered a measure of the rate of production. However, Smith and Brown (5, 6) found that soil respiration is not a simple diffusion of carbon dioxide from the soil and, therefore, is not an accurate measure of the rate of production.

Buckingham (1) made a study of the speed of diffusion of air and carbon dioxide into each other through the soil. He concluded that the speed of diffusion of carbon dioxide through the soil is proportional to the square of the percentage of free pore-space, and that the texture, structure, and moisture content do not influence it to any great extent. The mean diffusion constant for oxygen and carbon dioxide diffusing through 79 per cent nitrogen was computed and it was assumed that the same relation holds for the diffusion of carbon dioxide through the soil. Then, by means of this diffusion constant, the porosity of the soil, and the concentration of carbon dioxide in the soil air, the rate of production was calculated.

Buckingham recognized numerous sources of error in the determination of his diffusion constant, such as the thickness of soil, the analyses of the gases, and the regulation of pressures.

The purpose of the work reported here was to determine the relation between the rate of diffusion of carbon dioxide and the porosity of the soil, avoiding, if possible, some of the sources of error recognized by Buckingham and to ascertain what relation, if any, exists between the concentration of carbon dioxide in the soil, the rate of diffusion, and the respiration of the soil. The term "soil respiration" is used here as defined by Lundegardh (3).

[1] Journal Paper No. *B71* of the Iowa Agricultural Experiment Station.

EXPERIMENTAL

It was concluded that if the difficulties recognized by Buckingham and others were to be avoided, it would be necessary to maintain different partial pressures of carbon dioxide on two faces of a soil column and the same total pressure on each side, to obtain the column of soil as it exists in the field without disturbing its structure, and to make the analysis of the diffused gas directly.

Hannén (2) studied the rate of diffusion of carbon dioxide through soils of different texture. He placed a column of soil in one cylinder above another of the same size which was filled with pure carbon dioxide. After 10 hours the amount of carbon dioxide remaining in the lower cylinder was determined and the amount which had disappeared was assumed to have escaped by diffusion through the soil. In the work reported here, an effort was made to measure directly the actual amount of carbon dioxide diffusing through the soil.

An attempt was made to obtain the column of soil with structure unchanged by taking the sample from the field in a special apparatus. A brass cylinder 3 inches in diameter and 10 inches long (plate 1, fig. 1) was used for this purpose. The brass cylinder was made up in three sections and joined together by ground, flanged joints which fit tightly. The lower section was 1½ inches high and was sharpened to a cutting edge on the lower end. The top section was 2½ inches long and was fitted with a piece of solid cast iron for driving the cylinder into the ground. After the cylinder was driven into the ground it was dug out with a spade and carried into the laboratory, the top and bottom sections were removed, and the soil was sheared off smooth. The cylinder with the core of soil formed the center portion of the diffusion apparatus (plate 1, fig. 2). The lower chamber was 3 inches high and had two vents, an inlet tube for the gas mixture at the bottom and an outlet near the top of the section, or just below the core of soil, which was left open to the atmosphere. The top section of the apparatus was a chamber 3 inches high with two vents, one being at the bottom of the section, or just above the core of soil, to which a small U-tube manometer filled with water was attached, the other opening being at the top and used for taking samples of the gas for analysis. This section was also fitted with a stirrer to mix the gas before sampling. The apparatus was jointed together and tightly clamped by means of bolts with wing-nuts and a wooden clamp. The joints were coated with hot paraffin before being joined.

The concentration of carbon dioxide in the chamber above the soil was determined. A mixture of air and carbon dioxide of definite carbon dioxide content was passed slowly into the bottom chamber of the apparatus while the other opening near the bottom of the core of the soil was left open. Thus, air of a definite concentration of carbon dioxide was maintained on the lower face of the soil at atmospheric pressure. The air in the top cylinder was stirred, and samples were taken at intervals for analysis. The coefficient of diffusion, designated by K, is defined as the number of cubic centimeter of car-

bon dioxide at 0°C. and 760 mm. of mercury passing through 1 sq. cm. area of cross-section of soil 1 cm. thick per second when the partial pressures of CO_2 on the two faces of the soil differ by 1 mm. of mercury and the total pressure is 760 mm. The constant was calculated from the data according to the equation:

$$K = \frac{X\,H}{(C_1 - C_2)\,A\,t\,760}$$

in which X = The volume of carbon dioxide passing through the core of soil (0°C. 760 mm.).
H = The height of the column of soil in cm.
C_1 = The concentration of carbon dioxide on the lower face of the column of soil.
C_2 = The logarithmic average of initial and final concentrations of carbon dioxide on the upper face of the column of soil.
A = The area of cross section of soil, cm^2.
t = Time in seconds.

Since the diffusion of carbon dioxide through this column of soil depends upon the difference in concentration in carbon dioxide at the two faces of the core of soil and the diffusing gas is accumulated in the top chamber of the apparatus, the rate of diffusion will be decreased as the concentration of carbon dioxide in the chamber above the soil increases. The concentration of carbon dioxide in the chamber above the soil is constantly changing, hence initial and final concentrations were determined, and the logarithmic average was taken as the concentration for calculating the average rate of diffusion.

It was noted after the first experiment was performed, that carbon dioxide was being produced in the column of soil. This carbon dioxide produced during the experiment interfered with the determination and all attempts at sterilization of the soil without altering its structure failed. An attempt was made to determine the amount of carbon dioxide evolved during the experiment and correct for it in the calculation. Air was drawn through the soil by means of a vacuum pump at the rate of 10 liters per hour for 30 minutes, after which the concentration of carbon dioxide was determined in the chamber above the soil. A micro-Haldane gas analysis apparatus was used for the analysis. The concentration of carbon dioxide was determined again at 30-minute intervals for 2 hours and the amount of carbon dioxide evolved calculated. A constant rate of production being assumed, the amount of carbon dioxide produced during the experiment was subtracted from the total found in the chamber above the soil at the end of the experiment. The difference represented the amount passing through the soil by diffusion.

The porosity of the soil was determined as follows:

Weight of cylinder + moist soil	1,800 gm.
Weight of cylinder	870 gm.
Weight of moist soil	930 gm.
Weight of moisture	167 gm.
Weight of dry soil	763 gm.

Sp. Gr. of soil	2.24
Volume of soil	340 cc.
Volume of cylinder	577 cc.

$$\text{Porosity} = \frac{577 - (340 + 167)}{577} = 0.121$$

Diffusion of carbon dioxide through moist soil

A column of Carrington loam was taken from the Agronomy Farm and the rate of diffusion of carbon dioxide through the soil determined according to the

TABLE 1

Diffusion of carbon dioxide through moist soil

LENGTH OF DIFFUSION PERIOD	PERCENTAGE CO_2			PERCENTAGE INCREASE		$K \times 10^{-6}$
	Below soil	Above soil		Due to evolution of CO_2	Due to diffusion of CO_2	
		In beginning	After diffusion			
hour:minutes						
1:30	1.28	0.033	0.116	0.023	0.060	3.81
2:00	1.28	0.033	0.165	0.031	0.101	5.15
1:00	0.99	0.025	0.059	0.034	0.000	0.00
2:00	0.99	0.025	0.150	0.068	0.057	4.77
3:30	0.99	0.025	0.276	0.119	0.133	7.29
1:00	1.03	0.025	0.050	0.029	−0.004	0.00
2:00	1.03	0.025	0.150	0.058	0.067	5.12
1:00	1.22	0.024	0.082	0.026	0.032	3.31
2:00	1.22	0.024	0.173	0.052	0.097	5.35
4:00	1.22	0.024	0.346	0.104	0.218	5.29
1:00	0.536	0.040	0.041	0.024	−0.023	
2:30	0.536	0.040	0.208	0.061	0.107	21.8
1:00	1.545	0.049	0.049	0.024	−0.025	0.00
2:30	1.545	0.049	0.155	0.061	0.045	1.10
3:00	1.545	0.049	0.237	0.072	0.117	2.81
4:30	1.545	0.049	0.357	0.108	0.200	4.61

procedure outlined in the foregoing. Sixteen determinations were made, the length of the diffusion period varying from 1 to 4½ hours, and the diffusion constant was calculated. The results obtained appear in table 1.

The data in the table show that the average rate of diffusion increased with the length of diffusion time in all cases, except one, where it was approximately the same after 4 hours as after 2 hours. The factors influencing the rate of diffusion were the same for every determination, at least as nearly as it was

possible to maintain them under the conditions of the experiment. Thus, it would seem that the correction factor applied did not account for the amount of carbon dioxide produced in the soil during the period of the experiment. Thus, whereas the determination of the amount of carbon dioxide produced was made as accurately as possible, no account was taken of any of the gas passing into solution, adsorbed by the soil, or fixed in the soil. It appears

TABLE 2

Diffusion of carbon dioxide through air-dry soil ($K \times 10^{-7}$)

NUMBER OF DETERMINATIONS	POROSITY					
	0.364	0.426	0.512	0.518	0.625	0.645
1	1.99	1.91	1.72	1.89	2.58	2.90
2	3.11	1.79	1.92	2.99	3.00	3.19
3	1.83	0.89	2.16	3.26	2.66	2.29
4	2.33	1.01	3.38	2.81	3.43	2.53
5	2.25	2.36	1.73	1.54	2.41	2.35
6	2.75	2.14	2.94	2.67	2.97	3.46
7	2.25	3.98	2.85	3.50	2.67	3.66
8	2.55	1.13	2.89	1.77	2.21	3.06
9	2.60	2.72	2.26	2.49	0.77	4.05
10	2.60	2.13	2.85	2.73	2.76	2.29
11	1.10	2.25	2.57	3.55	2.78	2.31
12	1.65	3.25	3.17	3.35	2.14	3.87
13	2.44	2.62	....	....	1.08	2.77
14	....	2.68	....	....	2.15	3.54
15	....	3.15	....	....	3.46	3.99
16	....	3.09	....	....	2.18	5.05
17	....	2.67	....	....	2.24	3.73
18	....	2.55	....	....	2.95	2.97
19	....	2.28	....	....	1.77	3.70
20	....	2.71	....	....	1.92	4.53
21	....	2.61	....	....	2.08	3.24
22	....	....	....	....	1.96	....
23	....	....	....	....	2.88	....
24	....	....	....	....	2.52	....
25	....	....	....	....	3.19	....
Means....	2.265	2.377	2.537	2.713	2.430	3.309

that some of this carbon dioxide which cannot be accounted for may influence the rate of diffusion considerably. It is possible under conditions of rapid production of carbon dioxide to increase the concentration of carbon dioxide within the core of soil to a point where it would actually diffuse in both directions, that is, into both the upper and the lower chambers of the apparatus. The procedure was finally modified to consist of a determination of the average rate of diffusion through air-dry soil.

Diffusion of carbon dioxide through air-dry soil

A ¼-inch section of cylinder with fine-mesh brass screen was placed between the bottom chamber and the middle section of the cylinder. The air-dry soil was placed in this section and tamped lightly to compact the soil, and the apparatus was assembled. The porosity was then determined as in the previous work.

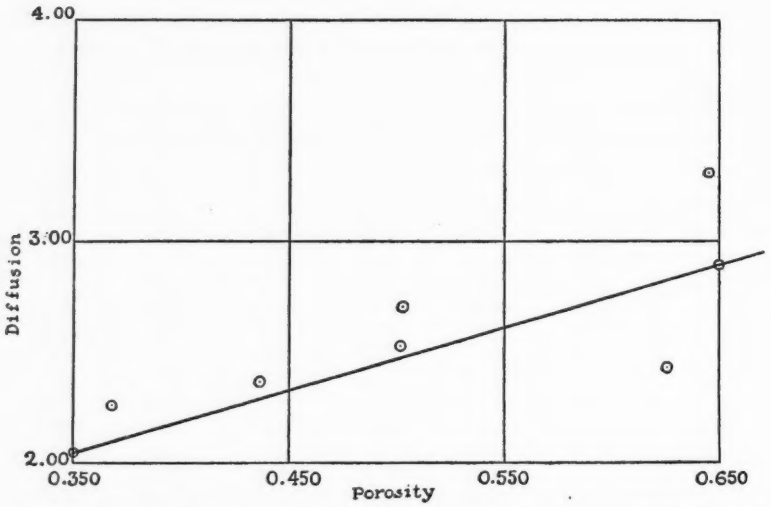

FIG. 1. REGRESSION LINE SHOWING THE RELATION OF RATE OF DIFFUSION OF CARBON DIOXIDE THROUGH AIR-DRY SOIL TO THE POROSITY OF THE SOIL

A large number of determinations on the rate of diffusion of carbon dioxide were made at 30-minute intervals. Six soils of different porosity were used. The results obtained are presented in table 2.

The data in the table show considerable variation within the porosity groups. However, the means of these groups, when tested by the method of analysis of variance[2] were found to vary significantly among themselves. That is, the average speed of diffusion of carbon dioxide through air-dry soil was found to be definitely related to the porosity of the soil.

Furthermore, it appears from the means in the table and from figure 1 that there is a trend toward higher rates of diffusion as the porosity increases. To test the significance of this trend, the correlation between rates of diffusion and porosity was computed and found to be 0.31, a highly significant value. The regression line, plotted in figure 1, has the equation,

$$\text{Dif.} \times 10^7 = 2.297\ (\text{porosity}) + 1.402$$

[2] Prof. G. W. Snedecor, statistical laboratory, Iowa State College, Ames, Iowa.

The regression coefficient indicates an average increase of 2.297×10^{-7} cc. carbon dioxide per unit increase in porosity, or 2.297×10^{-9} cc. for each per cent increase in pore space.

The diffusion of carbon dioxide through soils in situ and the relation to production and respiration

The average rate of diffusion of carbon dioxide through air-dry soil was found to be definitely related to the porosity of the soil. Inasmuch as the porosity of the soil is greatly altered by the structure of the soil and its moisture content, the rate of diffusion under field conditions would be expected to be very different from the average rate obtained with air-dry soil. Studies were made on the rate of diffusion of carbon dioxide through Carrington loam *in situ*. A respiration bell similar to the one used by Lundegardh (3) was used to collect the carbon dioxide evolved from the surface of a given area of soil. A tube (7) was placed in the soil at a depth of 15 cm. from which samples of soil air were taken for a determination of the concentration of carbon dioxide. The percentage of carbon dioxide in the bell air was determined in the beginning of the experiment and again after 20 to 30 minutes. The amount of carbon dioxide evolved was expressed as cubic centimeters at 25°C. and 760 mm. pressure. The rate of diffusion was calculated according to the equation:

$$\text{Diffusion} = \frac{R \cdot H}{(C_1 - C_2) \cdot A \cdot t \cdot 760}$$

in which R = The number of cc. carbon dioxide at 25°C. and 760 mm.
H = Depth of sample of soil air taken for analysis.
C_1 = Percentage of carbon dioxide in soil air at H.
C_2 = Logarithmic average percentage of carbon dioxide in the bell air from the beginning to the end of the experiment.
A = Area of soil under the bell.
t = Time in seconds.

The diffusion values obtained under these conditions would not, of course, represent the rate of diffusion, since carbon dioxide is being produced in the soil while the measurements are being made. The results may, however, be of value as an indicator of soil moisture conditions, aeration, and, in fallow soils, as an index of microbiological activity.

The respiration and the rate of production of carbon dioxide in this soil were calculated from the measurements made, and the results were expressed as milligrams carbon dioxide per square meter per hour. The rate of production was calculated by assuming the simple relation, production = concentration (CO_2 pressure gradient per centimeter) times diffusion. The coefficient of diffusion being that for air-dry Carrington loam. The average moisture content was about 15 per cent and the free pore-space was 0.350. The results obtained are presented in table 3.

The data in the table show a considerable variation in the percentage of carbon dioxide in the soil air at a depth of 15 cm. from August 3 to September

10. The respiration rate varied widely during the same period but showed no apparent relation to the concentration of carbon dioxide in the soil air. The diffusion value obtained by calculation from the concentration of carbon dioxide and the respiration, a resultant of production, solution, and diffusion, is perhaps a more reliable index of microbiological action than either concentration or respiration alone. It is, however, difficult to obtain representative samples of the air under the bell for analysis. The inaccuracies in the measurement of the amount of carbon dioxide evolved from the soil are reflected in the diffusion value. Furthermore, the numbers mean nothing in so far as showing anything about the rate of diffusion of carbon dioxide through the soil is concerned.

The rate of production as calculated from the concentration of carbon dioxide in the soil air was much lower than the respiration. These results show the

TABLE 3

The relation of concentration of CO_2 in soil air to rate of production, diffusion, and respiration

DATE (1931)	PERCENTAGE CO_2 IN SOIL AIR AT 15 CM. DEPTH	DIFFUSION $\times 10^{-5}$	RESPIRATION (MGM. CO_2 PER SQ.M. PER HR.)	RATE OF CO_2 PRODUCTION (MGM. PER SQ.M. PER HR.)
August 3	1.00	3.358	81.75	3.94
August 5	0.89	2.865	66.83	3.77
August 6	0.87	2.577	49.89	3.35
August 7*	0.88	2.639	53.25	3.21
August 10	0.54	0.719	9.37	2.00
August 21	0.60	2.535	39.40	2.19
August 24	0.93	32.750	826.70	3.68
August 25	0.88	14.100	296.30	2.93
August 27	0.63	3.560	52.75	2.42
September 2	0.66	2.957	52.84	2.93
September 10	0.30	8.832	14.66	1.16

* Raining.

same variation as the percentage of carbon dioxide in the soil air, are based on static or constant conditions which are not obtainable in the soil, and cannot, therefore, be regarded as a reliable measure of the rate of production of carbon dioxide in the soil.

SUMMARY AND CONCLUSIONS

The diffusion of carbon dioxide through moist soil was found to be so complicated by the production of carbon dioxide in the soil while the measurements were being carried out that an accurate determination of the rate of diffusion could not be made. This, no doubt, explains why Buckingham found diffusion to be an exponential function of porosity and concluded that diffusion was not influenced to any great extent by moisture, texture, and structure. These factors do influence the rate of diffusion, at least in so far as they affect porosity,

since diffusion is a function of porosity. An effort was made to estimate the amount of carbon dioxide produced during the experiment and correct for it in the calculation of the diffusion coefficient. Finally, however, this variable was eliminated by using air-dry soil. More than 100 experiments were made, using air-dry soils differing in porosity. It was concluded from the data obtained that the rate of diffusion of carbon dioxide through air-dry soil is a linear function of porosity of the soil within the limits of porosity studied. This does not mean, however, that at zero porosity, that is, soil saturated with water, that there would be no diffusion of carbon dioxide through the soil; nor that at 100 per cent porosity, that is, free diffusion, this relation would hold.

The diffusion of carbon dioxide through Carrington loam *in situ* was determined 11 times over a period of about 5 weeks. The results obtained varied from 2.535×10^{-8} to 32.750×10^{-8}. Since the method is subject to certain inaccuracies, caution must be observed in the interpretation of the data. However, since it is a resultant of many (4) forces some of which are related to the production of carbon dioxide in the soil, it would seem to be a better index of the amount of carbon dioxide produced in soils *in situ* than either the concentration of carbon dioxide in the soil air or the evolution of carbon dioxide from the soil alone.

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PLATE 1

CARBON DIOXIDE DIFFUSION APPARATUS

FIG. 1. Parts of diffusion apparatus

FIG. 2. Diffusion apparatus assembled

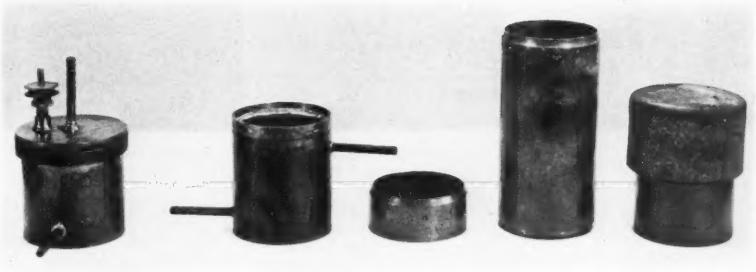

FIG. 1

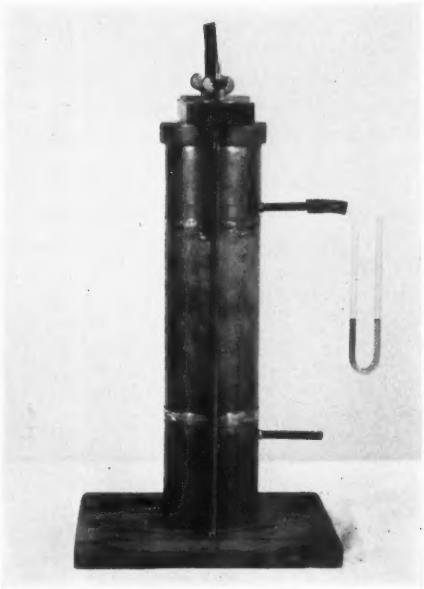

FIG. 2

THE IODINE CONTENT OF THE SOIL IN KENTUCKY[1]

J. S. McHARGUE AND D. W. YOUNG

Kentucky Agricultural Experiment Station

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The importance of iodine in the metabolism of animals was established only recently through the researches of Baumann (1) who, in 1895, proved that iodine is localized in the thyroid gland. In 1915, Kendall (4) demonstrated that the thyroid gland secretes the hormone thyroxine, which contains 65 per cent of iodine. That the presence of a small but adequate quantity of iodine in the organism is necessary to the physiological well-being of man, livestock, and plants is now widely recognized. The iodine contained in the bodies of animals is absorbed from the food and water ingested; that in plants, if we exclude fertilizers, comes from the soil. It is therefore of fundamental importance in the sciences of agriculture, medicine, and nutrition, to ascertain whether the soil of any region which produces foods is adequately supplied with iodine. Thus far only a few investigators have determined iodine in soils, and these investigations, for the most part, were carried on in foreign countries.

REVIEW OF PREVIOUS WORK

About 1850, Chatin (3) first demonstrated that small quantities of iodine are widely distributed in nature. He also pointed out that iodine is not equally distributed in natural waters, rocks, and soils and that endemic goiter is associated with certain areas of soils which are low or deficient in iodine, in his country. Chatin's theory, that iodine-deficient foods and waters are a cause of endemic goiter, was not accepted by his contemporaries at the French Academy of Science, where his paper was presented; however, the researches of a number of investigators in recent years have sustained its correctness.

Benson and Carter (2) published analyses showing the iodine content of soils from Western Australia, New Zealand, and adjacent islands. The maximum iodine content of six soils from Australia was 9.6 p.p.m.: the minimum, 0.7 p.p.m., and the average, 3.53 p.p.m. Five samples of soil from islands near by, derived from basaltic rocks, contained about 10 times as much iodine as the average of the soils from Australia.

Shore and Andrew (7) reported the iodine content of 65 soils collected in several districts of the North Island and from other islands adjacent to the New Zealand group. The maximum finding was 24 p.p.m.; the minimum, 1 p.p.m., and the average, 8.22 p.p.m.

Orr and Leitch (6) state that the amount of iodine found in soils ranges from 0.6 to 6.0 p.p.m., though amounts outside of these limits may be found. Soils from England and Scotland, analyzed at the Rowett Institute, contained from 2.4 to 8.0 p.p.m. of iodine; two soils from Kenya contained 3.0 and 3.5 p.p.m.; and one soil from the Falkland Islands contained 25.0 p.p.m. It is quite probable that the soil from Falkland Island, as well as that from

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the director.

the New Zealand group, which contained a similar amount of iodine, has been enriched in iodine by the excreta and debris from the large flocks of sea birds which inhabit some of the islands and probably have done so for many years.

EXPERIMENTAL DATA

Figure 1 is an outline map of Kentucky showing the location and the approximate size of the six areas studied. Starting at the left these are numbered from 1 to 6.

The samples of soil used in this investigation were collected at different times in the past 20 years and have been kept in an air-dry condition, in closed glass jars, in the department of chemistry of the Kentucky Agricultural Experiment Station.

Method of determination

The combustion method used for the determination of iodine in the soils was that recently published by McHargue, Young, and Roy (5). In tables 1 to 6,

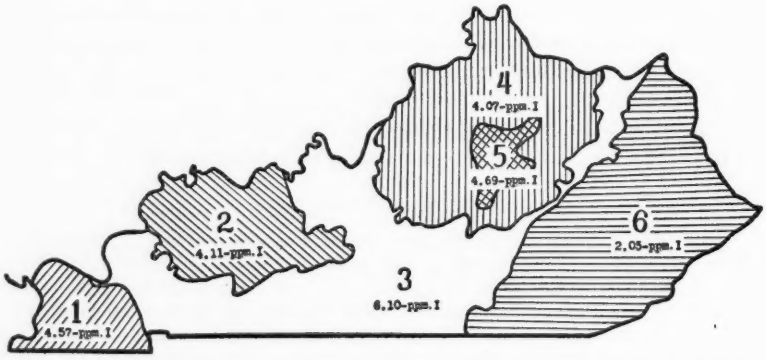

FIG. 1. OUTLINE MAP OF KENTUCKY SHOWING LOCATION AND APPROXIMATE SIZE OF AREAS IN WHICH IODINE CONTENT OF SOILS WAS DETERMINED

8, and 9, each result for iodine by combustion is the mean of two independent combustions of 10 to 100 gm. of soil. If the duplicate determinations were not consistent, a third was made and the mean of the two closest results taken. Agreement within about 8 per cent of the smaller figure was considered satisfactory.

The results under the heading "fusion" are single determinations, obtained by fusing 5 to 10 gm. of soil with potassium hydroxide in a large iron crucible, as in von Fellenberg's method, though the subsequent treatment was different from that described by von Fellenberg. The iodine was extracted from the salts with 95 per cent alcohol, liberated with sulfuric acid and sodium nitrite, and extracted with carbon disulfide, which was centrifuged and the solution compared by means of a micro-colorimeter, with a standard prepared in a similar way. All reagents were proved free from iodine before use.

Area 1—the Purchase

Area 1 is that part of the state which lies west of the Tennessee River, locally known as the "Purchase." It includes 8 counties, contains about 2,400 square miles and has a population of approximately 150,000. The soils are silt loams, underlaid by beds of gravel, sand, and clay of Quaternary, Tertiary, and Cretaceous age. The surface soil of a considerable part is loess. The average iodine content of the 42 samples, from 7 counties, is 4.57 p.p.m.;[2] the maximum, 6.93; and the minimum, 1.59, as determined by the combustion method. From these it appears that the soil of the Purchase area is fairly well supplied with iodine. Detailed results are contained in table 1.

TABLE 1

Iodine content as parts per million of air-dry soil from area 1—the purchase region

COUNTY	COMBUSTION			FUSION
	Number of samples analyzed	Average	Range	Single determinations
		p.p.m.	*p.p.m.*	*p.p.m.*
Ballard	7	4.77	2.50–6.03	5.00
Carlisle	2	3.00	2.35–3.65	....
Fulton	8	4.74	2.60–6.35	5.90
Graves	6	5.34	4.65–6.93	5.43
Hickman	5	4.34	2.07–5.75	4.80
Marshall	9	4.08	1.59–5.70	5.90
McCracken	5	4.85	4.18–5.78	5.90
General average	42	4.45	1.59–6.93	5.49*

* Six determinations.

Area 2—the Western Coal Field

Area 2 contains approximately 4,850 square miles, in 11 counties, and has a population of about 265,700. The soils were derived largely from the disintegration of sandstones, shales, and clays, of Pennsylvania age, mostly in place. Some loess is present. Silt loams predominate. Sixty-nine samples, from 9 of the 11 counties in the area, were analyzed by the combustion method. The average iodine content of these is 4.11 p. p. m.; the maximum, 7.37, in a sample from Muhlenberg County; the minimum, 2.31, in one from Union County. The average for this area is slightly lower than that for the Purchase area. Table 2 contains the results in detail.

Area 3—Mississippian Area

The Mississippian area surrounds the Western Coal Field on the west, south, and east and extends to the western border of the Eastern Coal Field. The rocks are limestones, shales, and sandstones of Mississippian age. It contains

[2] To save space and for simplicity, the findings are stated as parts per million. The results by previous investigators usually have been stated in parts per billion.

approximately 11,667 square miles, in about 28 counties, and a population of approximately 455,100. The soils were derived largely from the disintegration

TABLE 2

Iodine content as parts per million of air-dry soil from area 2—the Western Coal Field

COUNTY	COMBUSTION			FUSION
	Number of samples analyzed	Average	Range	Single determinations
		p.p.m.	*p.p.m.*	*p.p.m.*
Butler	4	4.31	4.10–4.76	3.40
Daviess	10	3.25	2.67–4.15	3.66
Henderson	10	5.20	4.30–6.87	6.50
Hopkins	7	4.00	3.61–4.41	4.58
McLean	4	3.39	2.91–3.80	3.28
Muhlenberg	10	4.90	3.92–7.37	6.10
Ohio	9	4.31	3.75–4.95	4.80
Union	6	3.07	2.31–3.75	2.50
Webster	9	3.77	3.35–4.21	3.60
General average	69	4.02	2.31–7.37	4.27*

* Nine determinations.

TABLE 3

Iodine content as parts per million of air-dry soil from area 3—the Mississippian

COUNTY	COMBUSTION			FUSION
	Number of samples analyzed	Average	Range	Single determinations
		p.p.m.	*p.p.m.*	*p.p.m.*
Adair	8	5.80	4.00– 6.85	6.50
Barren	10	6.68	4.88– 8.75	7.30
Christian	12	6.80	3.98–16.70	4.57
Green	4	5.55	4.45– 6.15	6.50
Hart	9	6.48	5.17– 8.34	8.63
Hardin	5	5.43	4.68– 7.12	5.40
Larue	3	4.44	3.00– 5.80	4.80
Livingston	6	4.90	4.36– 5.80	5.50
Logan	9	9.88	3.60–16.95	3.80
Lyon	7	4.11	2.70– 4.98	3.00
Meade	6	4.26	3.45– 6.08	4.90
Metcalfe	6	4.38	3.50– 5.26	4.20
Taylor	2	6.64	6.30– 6.98	....
Todd	6	7.74	6.10– 8.88	9.60
Warren	7	5.16	2.53– 8.38	6.00
General average	100	6.10	2.53–16.95	5.76*

* Fourteen determinations.

of limestone rocks, in place, and they are generally silt and clay loams. One hundred samples, from 15 counties, were analyzed by the combustion method.

The average iodine content is 6.10 p.p.m.; the maximum, 16.95, in a sample from Logan County; the minimum, 2.53, in one from Warren County. This area gives a considerably larger general average for iodine than the others. The reason is probably because the red loam soil, derived from limestone, predominates in this area. The high results, 11, 12, and 16 p.p.m., indicate that a type of soil high in iodine occurs in this part of the state. The detailed results are stated in table 3.

TABLE 4

Iodine content as parts per million of air-dry soil from area 4—the Outer Bluegrass Region

COUNTY	COMBUSTION			FUSION
	Number of samples analyzed	Average	Range	Single determinations
		p.p.m.	*p.p.m.*	*p.p.m.*
Bath	9	3.93	3.16– 4.85	5.30
Boone	7	2.83	2.25– 3.34	3.10
Bracken	4	4.53	4.20– 4.85	5.29
Campbell	7	6.22	5.55– 7.00	6.00
Grant	5	4.19	3.25– 4.55	5.00
Harrison	11	3.99	3.10– 4.90	3.50
Henry	9	2.71	1.10– 4.85	4.80
Kenton	11	3.41	1.15– 4.29	4.10
Lincoln	2	5.54	5.45– 5.62	....
Madison	10	2.09	1.15– 4.25	4.50 2.60
Montgomery	10	3.95	2.95– 4.75	4.20
Nicholas	7	3.71	2.95– 4.85	5.27
Owen	2	4.85	4.55– 5.15	....
Pendleton	9	4.65	3.71– 6.10	6.32
Robertson	2	4.10	3.75– 4.45	....
Shelby	13	3.59	1.95– 6.00	4.01
Spencer	8	8.68	4.85–11.85	5.40
Washington	6	3.48	3.10– 4.10	4.50
General average	132	4.24	1.10–11.85	4.50*

* Sixteen determinations.

Area 4—the Outer Bluegrass Region

Area 4 occupies the north central part of the state and contains approximately 6,722 square miles and a population of approximately 480,200. The soils were derived mainly from the disintegration, in place, of limestones and shales of Cincinnatian age. They are mostly silt and clay loams. One hundred and thirty-two samples, from 18 of the 30 counties in this area, were analyzed by the combustion method. The average iodine content is 4.07 p.p.m.; the maximum, 11.85, in a sample from Spencer County; the minimum, 1.10, in one from Henry County. The average for Spencer County, 8.68 p.p.m., is the second highest for any county in the state. The soils of this area are fairly well supplied with iodine but the element appears to be less abundant than in the soils of the Mississippian area. The findings are given in detail in table 4.

Area 5—the Inner Bluegrass Region

Area 5 occupies the south central part of the Bluegrass Region. It is also called the "Bluegrass Region proper," or the "Central Bluegrass Region." The area is characterized by the occurrence of highly phosphatic soil derived from limestone of the Lexington formation. Parts of some of the counties included here, however, do not belong to the Inner Bluegrass Region proper. Area 5 contains approximately 1,750 square miles and has a population of about 156,600. Most of the soils were formed in place, mainly from limestone strata, and silt loams and clay loams predominate. Sixty-four samples, from the 7 counties, were analyzed by the combustion method. The average iodine content is 4.69 p.p.m.; the maximum, 8.25, in a sample from Mercer County; and the minimum, 2.40, in one from Bourbon County. The average is slightly

TABLE 5

Iodine content as parts per million of air-dry soil from area 5—the Inner Bluegrass Region

COUNTY	COMBUSTION			FUSION
	Number of samples analyzed	Average	Range	Single determinations
		p.p.m.	*p.p.m.*	*p.p.m.*
Bourbon	9	3.80	2.40–5.25	4.40
Clark	9	3.18	2.65–3.60	3.50
Fayette	14	5.45	3.65–7.29	6.20 4.80
Jessamine	7	4.00	3.16–4.46	3.50
Mercer	9	5.45	3.14–8.25	5.63
Scott	6	4.08	3.49–4.59	3.90
Woodford	10	3.77	2.05–4.50	4.10
General average	64	4.35	2.05–8.25	4.50*

* Eight determinations.

larger than that for the soils of the outer bluegrass area. Because much of the soil of the central area was derived from the disintegration of phosphatic limestone, higher results for iodine were anticipated.

Area 6—the Eastern Coal Field

Area 6 forms the eastern part of the state and contains about 8,760 square miles, of hilly to mountainous topography. The tallest peaks exceed 4,000 feet above sea level. The population is approximately 638,500. The soils are sandy loams to silt loams, in the main, derived from the disintegration of sandstones and shales of Pennsylvanian age.

Only 14 samples, from 6 counties, were available for iodine determination. The maximum iodine content found by the combustion method is 3.08 p.p.m., in a sample from Pike County; the minimum, 0.81, in one from McCreary

County. The average for the 14 samples is 2.05 p.p.m., which is about half the average of the soils in the Western Coal Field area. It is therefore evident that the mountainous area contains a smaller amount of iodine in the soil than any of the other areas. Although the number of samples analyzed for iodine was smaller than in any other area of near the same size, the samples were taken in the proper manner and it is believed that they give a fair representation of the iodine content of the soils of this part of the state.

TABLE 6

Iodine content as parts per million of the air-dry soil of area 6—the Eastern Coal Field

COUNTY	COMBUSTION			FUSION
	Number of samples analyzed	Average	Range	Single determinations
		p.p.m.	*p.p.m.*	*p.p.m.*
Lawrence	3	2.14	1.40–2.53	2.46
Leslie	1	2.23	.....	1.95
Magoffin	1	1.58	.....	1.75
Martin	2	2.15	1.87–2.42	2.50
McCreary	1	0.81	.....	....
Pike	6	2.24	1.50–3.08	2.00
General average	14	2.05	1.40–3.08	2.13*

* Five determinations.

TABLE 7

Summary of the iodine content of soil from the six areas, stated as parts per million of air-dried soil

AREA	Number of counties represented	Number of samples analyzed	IODINE CONTENT		
			Maximum	Minimum	Average
			p.p.m.	*p.p.m.*	*p.p.m.*
Area 1—Purchase	7	42	6.93	1.59	4.57
Area 2—Western Coal Field	9	69	7.37	2.31	4.11
Area 3—Mississippian	15	100	16.95	2.53	6.10
Area 4—Outer Bluegrass Region	18	132	11.85	1.10	4.07
Area 5—Inner Bluegrass Region	7	64	8.25	2.40	4.35
Area 6—Eastern Coal Field	6	14	3.08	0.81	2.05
All areas	62	421	16.95	0.81	4.59

Tables 8 and 9 contain the results for iodine determined by the combustion method in samples of soil taken to represent the different soil horizons of the Maury and Mercer silt loam types, respectively. The Maury soil was derived from the disintegration of phosphatic limestones and the phosphorus content increases more or less uniformly from 0.33 per cent in the surface soil to about

10.5 per cent in the lowest stratum. The surface layer contains the largest amount of iodine and the subsequent layers vary irregularly in iodine as the depth increases. The average for the whole profile is 5.16 p.p.m.

TABLE 8

Iodine content of Fayette County soil, Maury type, taken at different depths, air-dry

NUMBER	SOIL HORIZON	DEPTH	CHARACTER	IODINE
		inches		*p.p.m.*
422	A 1	0–8	Brown silt loam	8.95
423	A 2	8–17	Reddish brown silt loam	6.60
424	B 1	17–34	Reddish brown silty clay loam	4.40
425	B 2	34–66	Silty clay loam	4.00
426	C 1	66–72	Dark chocolate-brown	7.70
427	C 2	72–86	Very dark chocloate-brown. Semi-weathered	5.80
428	C 3	86–104	Dark brown silty clay	4.40
429	C 4a	104–x	Dark chocolate-brown silty clay	4.05
430	C 4b	x–130	Like C 4a	4.00
431	C 5	130–144	Blackish, gritty material	7.00
		Rock		
Average*				5.16

* Computed on the assumption that the weight of soil in each division is proportional to its thickness.

TABLE 9

Iodine content of Fayette county soil, Mercer silt loam type, taken at different depths, air-dry

NUMBER	SOIL HORIZON	DEPTH	CHARACTER	IODINE
		inches		*p.p.m.*
432	A_1	0–7	Dull brown silt loam	6.00
433	A_2	7–20	Rusty brown silt loam	4.70
434	A_3	20–36	Granular silt loam	5.00
435	B_1	36–54	Brown and rusty yellow clay	6.70
436	B_2	54–76	Bluish and pale yellow clay	9.70
437	C_1	76–86	Blue, gray rusty brown clay	4.70
438	C_2	86–96	Blue, yellow and brown clay	4.00
439	C_3	96–116	Yellow clay (alkaline)	6.30
Average*				6.29

* Computed on the assumption that the weight of soil in each division is proportional to its thickness.

Samples of limestone from a fresh excavation near the place where the samples of soil for the Maury profile were taken, were analyzed for iodine. A sample from near the top of the underlying rock contained 0.25 p.p.m. of iodine. A fossiliferous layer about 3 feet lower contained 2.0 p.p.m. of iodine. There-

fore, iodine occurs in greater concentration in soil than in the underlying limestone from which the soil was derived.

Plates of phosphate rock taken from soil immediately above the underlying limestone, were analyzed for phosphorus and iodine. The sample contained 75.0 per cent of tricalcium phosphate and 20 p.p.m. of iodine. The high result for iodine in the phosphate indicates that this material contains an iodine mineral, possibly similar in composition to appatite, which mineral contains fluorine and is associated with phosphate rock.

The Mercer silt loam soil was derived from limestone low in phosphorus. The maximum phosphorus content of the soil of this profile is approximately 0.3 per cent and the minimum, 0.1 per cent. The maximum iodine content of the Mercer silt loam, 9.7 p.p.m., occurred below the middle of the profile, and the minimum, 4 p.p.m., still lower. The average for the profile was 6.29 p.p.m. The results for iodine in the two soil profiles show that there is no close correlation between iodine content and phosphorus content in these two soils.

DISCUSSION

Fusion analyses of 58 samples of soil from as many counties averaged 4.70 parts of iodine per million of dry soil, whereas combustion analyses of the same samples averaged 4.44 p.p.m. The result is confirmatory of the findings by the combustion method.

It is to be observed that the soils in area 3 contain the largest quantity of iodine and those in area 6 the smallest. However, an inspection of the individual results in tables 1 to 6 shows that a few low results are contained in each, which is an indication that each area contains soils that are low and possibly deficient in iodine, from the standpoint of agriculture. On the other hand, there are a few soils in the state that are exceptionally rich in iodine. How extensive the high and low iodine areas are remains to be ascertained.

The soils of the Purchase Region are unique inasmuch as they were formed in a different way from those of the other five areas. Though largely derived from powdered rock material which was carried to its present location by streams of fresh water and deposited in an ancient embayment, they contain more loessal material supposed to have been brought by the wind from a westerly direction, than those of any other area. The water-borne débris has undergone considerable weathering and probably has lost a part of the iodine which it contained at the time it was eroded from the rock. The fact that the soils of this area rank third among those of the other geological areas of the state in iodine content is of considerable interest and suggests that possibly the soil material was eroded from rocks containing considerable iodine a part of which they have retained until the present time.

The soils of the Western and Eastern Coal Field areas were derived mainly from the disintegration of sandstone and shale strata in place. The soils of the Eastern Coal Field contain about half as much iodine as those in the Western Coal Field. Apparently the soils of mountainous regions elsewhere

have been found to contain less iodine than those at lower altitudes. The soils of the Eastern Coal Field seem to follow this general rule. Because the soils of the Eastern Coal Field are low in iodine we would expect a greater incidence of goiter in this area than elsewhere in the state, as well as of those affections of livestock which attend iodine deficiency.

The soils of the other three areas have a general relationship in that they were derived mainly from the disintegration of limestone strata. Soils derived from limestone received their iodine from marine sources rather than from particles of undecomposed rock and probably have retained it by adsorption. The sea has long been regarded as the principal source of iodine. However, Orr and Leitch (6) point out that the iodine content of soils ranges from 0.6 to 6.0 p.p.m. and that the earth's crust, and not the sea, is the principal storehouse of iodine.

It seems reasonable to suppose that as particles of calcium and magnesium carbonate were precipitated in sea water they adsorbed and held tenaceously, molecules of some iodine compound derived from the débris from marine organisms and plants which was rich in iodine; accordingly, limestone strata contain more or less iodine, and the amount in Kentucky limestones appears to vary with the purity of the strata, the crystalline deposits containing the smallest quantity. Orr and Leitch (6) present data which show that fossiliferous limestones contain nearly twice as much iodine as non-fossiliferous deposits. Soils derived from the former should contain a larger quantity of iodine. However, they state that a wide range of variation in iodine content exists within each formation and that no sharp distinction can be drawn between the amount of iodine contained in different strata. Hence rocks belonging to the same formation from different districts may differ widely in their iodine content.

As the limestone strata weather from the top surface, beneath the soil, small quantities of iodine compounds may go into solution and may be either carried away in drainage waters or left in the débris, adsorbed by particles of clay and iron oxide. An iodine compound that resists weathering, such as that in the phosphate plates, would remain. Consequently, the disintegration of limestone should give rise to soils which contain considerable quantities of iodine. This may account for the high iodine content of some of the soil samples from the Mississippian, Outer Bluegrass, and Lexington areas in Kentucky. Then, too, the red clay soils which predominate in the Mississippian area and the other areas of limestone origin probably have peculiar adsorptive properties due to the hydrous ferric oxide which they contain in considerable amount.

SUMMARY

Four hundred and thirty-nine samples of soil from the six principal geological areas in Kentucky were analyzed for iodine. The largest quantities were found to be associated with soils derived from limestone strata. The smallest quantity of iodine occurred in the soils derived from sandstone strata, in the Eastern Coal Field area. It therefore appears that the foods and natural waters produced in some parts of the Eastern Coal Field may be deficient in

iodine to the extent that endemic goiter is more likely to be prevalent in this part of the state than in any other part. The soils of the Western Coal Field area contain nearly twice as much iodine as those of the Eastern Coal Field. The iodine content of the Maury (phosphatic) and the Mercer (non-phosphatic) soil profiles in Fayette County show considerable variation at different depths. There was no correlation between the phosphorus and iodine content in the two profiles. Soils derived from limestone rocks contain considerably more iodine than the unaltered rock.

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STUDIES ON READILY SOLUBLE PHOSPHATE IN SOILS: I. EXTRACTION OF READILY SOLUBLE PHOSPHATE FROM SOILS BY MEANS OF DILUTE ACID POTASSIUM SULFATE ($KHSO_4$)[1]

H. W. LOHSE AND G. N. RUHNKE[2]

Ontario Agricultural College[3]

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Many different methods have been proposed for the extraction of phosphate from soils by means of dilute acids in order to get an estimate of their fertility in respect to phosphate. One group of these methods is based upon the different hypothesis dealing with the plant's absorption of nutrients from the soil, and in one way or another the scope has been to approximate this absorption. Another group of such methods is based upon chemical considerations, and it is often assumed that the compounds in the soil are of the same constitution and nature as the common known synthetic phosphate compounds. It seems logical to assume that the usefulness of any chemical fertility method is mostly dependent upon how the results can be interpreted. If, by an extraction method, results can be obtained which are on a uniform basis in respect to the most important factors determining the solubility of the nutrients, for the case here—of phosphate, it is to be expected that the results will be interpretable. The researches presented in this paper are part of a series of researches on phosphates started in July, 1931.

REVIEW OF LITERATURE

Comprehensive investigations of Hoagland and his co-workers (16, 18, 17, 43) on the plant's intake of nutrients from the medium of growth have fully shown that the laws governing these processes are much more complicated than is usually assumed by the chemists who have proposed methods on such a basis.

It is an important fact, however, that the solubility of the compounds of N, P, and K in the medium of growth and the amounts taken up by the plants are rather closely correlated. In general, the more easily soluble N, P, and K there are in the medium of growth, the higher is the total content of these nutrients in the crop (1, 19).

That the plants, to some extent, can very likely get their supply of phosphate by direct action on the solid soil phase has been pointed out in a very logical way by several investigators (5, 24, 28, 30). Thus, Comber emphasizes that the soil and the plant form one sys-

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[2] Research assistant and assistant professor, respectively. The authors are indebted to Dr. H. D. Chapman, University of California, Citrus Experiment Station, Riverside, California, for valuable suggestions for the technic of the colorimetric method used. Acknowledgements are also due to Mr. G. B. Whiteside, of this department, for drawing the curves.

[3] Published with the permission of the president.

tem as the root hairs and the soil particles are cemented together. This offers the possibility that the cell sap might have dissolving action directly on the soil particles, and thus both inorganic and organic compounds in the cell sap may account for the dissolving action of the plant roots on the soil compounds. The fact that the phosphates common in soils (under the acidity conditions prevailing in many soils) may be entirely precipitated from the soil solution (11) supports the assumption that the plant roots very likely have a directly dissolving action on the solid soil phase. For the case of calcareous soils, solvents other than carbon dioxide must be of major importance in dissolving the soil phosphates (33). McGeorge and Breazeale (23) have shown that black alkali soils which contain sufficient replaceable Na to yield free hydroxyl ions on hydrolysis, contain readily soluble phosphate in large amounts, but the high alkalinity checks its absorption by the plants. Some investigations also indicate that there may be more water-soluble phosphate in some soils than the plants assimilate (7, 39).

From the literature reviewed here, it becomes apparent, that an estimation of the amount of *available* phosphate in a soil offers considerable difficulties.

Rindell (29) long ago called attention to the importance of a more detailed control in chemical studies on products as soils and the like. The same author recognized such problems as re-precipitation of dissolved phosphate in phosphate extractions, acidity control of the extracts, and the importance of the ratio of the solid mass to the solvent.

Very comprehensive studies on the solubility of soil phosphates by von Wrangell and her co-workers (39, 40, 41, 42) have revealed that the soil phosphates must be largely of a very complex nature, and their solubility is far less than that of the synthetic tertiary Ca- and Mg-phosphates and the synthetic phosphates of Al and Fe.[4] Wrangell also points out that the laws of diffusion do not govern the dissolution rate of these phosphates, but the dissolution rate is governed by the rate of the decomposition of the surface of the particles which contain the phosphate. Gregoire (13) concludes that the water-soluble soil phosphate mainly originates from colloidal compounds.

Hibbard (15), on the basis of a comprehensive study of the solubility of soil phosphates in different solvents, has come to the conclusion that the amount of phosphate dissolved in acid extract increases when the hydrogen-ion concentration and the relative volume of the solution increases. By extracting soils with a large number of neutral salt solutions, appreciable amounts of phosphate only were dissolved by using ammonium salts of oxalic and citric acid.

The effectiveness of acid solvents is obviously due to the small volume of the hydrogen ion which, because of this, is able to enter into very fine openings, and it can also come into closer proximity with negatively charged particles and be held more firmly than any other positive ion (26). The capillary and porous structure of the soil colloids is, no doubt, very important for their disintegration (14, 25, 35).

The solubility of the phosphates commonly existing in soils is mainly determined by the acidity of solution (2, 8, 9, 11, 35). In the figures obtained in these investigations, some minor disagreements exist, due to the different experimental methods used. The investigations reveal, however, that the different phosphates have a distinctive range of minimum solubility. When the bases used in soils are in excess, the phosphates can be almost completely precipitated from their solutions. The following figures from Gaarder's detailed investigations (11) give an illustrative picture of the behavior of some of the most important phosphates found in soils.

	pH range of minimum solubility
Excess of Fe-hydroxide	3 –4
Excess of Al-hydroxide	5 –6
Excess of Al-hydroxide + Ca	5 –6.3
Excess of Al-hydroxide + Mg	4.6–6.5

[4] Fe means in this paper Fe^{III}.

In these pH ranges, the phosphates are practically completely precipitated from their solutions.

The studies on the precipitation of phosphates explain the fact that re-precipitation of dissolved phosphate may take place during extraction of phosphate from soils if the soil remains in contact with the extract for a long time and the acidity of the solvent is below about a pH of 3. That such re-precipitation actually takes place in extraction of soil phosphate with dilute acids becomes apparent from the studies on the action of dilute acids on phosphorus compounds in soils by Russell and Prescott (32). Re-precipitation was most marked for dilute HNO_3, less for HCl, and least for H_2SO_4. This phenomenon was recognized by the fact that short-time extractions often yielded more phosphate to the solutions than did long-time extractions, and it was later proved by Comber (6).[5]

METHODS OF EXPERIMENTATION

To determine the small amounts of phosphate in soil extracts, the Deniges colorimetric method, as worked out by Truog and Meyer (37) and somewhat modified by Chapman (4), has been used.

The extraction method of Truog (36) for extracting "readily available" phosphate from soils has been used in some of the experiments. By this extraction method a solvent of 0.002 *N* H_2SO_4 buffered with $(NH_4)_2SO_4$ to a pH of 3 is used. Two grams of soil is shaken with 400 cc. solvent for half an hour.

If not otherwise stated, air-dried soil passed through a 20-mesh sieve is used. The pH determinations in all extracts are made by means of the quinhydrone electrode. For soil suspensions, both the quinhydrone electrode and the colorimetric methods have been used. All extractions were made at a temperature of 20 to 22°C.

EXPERIMENTAL DATA

The purpose of the experimental work carried out has been to find a suitable inorganic acid solvent for extraction of the more readily soluble phosphate from soils, and to work out a technic for such extractions.

Experiments with a series of different solvents revealed that a solvent of dilute acid potassium sulfate ($KHSO_4$) possesses distinct qualities for the purpose of extraction of readily soluble soil phosphate.[6] This solvent is, in concentrations of 0.01 *M* and upwards, appreciably buffered as a result of the partial dissociation of the HSO_4-ion.

The salt measured was *Potassium Bisulfate Merck C.P. Crystals* (*$KHSO_4$*). As the salt is deliquescent, itwas dried at 33–35°C. for one week. The pH values were made by means of a quinhydrone electrode using a saturated

[5] Comber (6) showed that the velocity of dissolution of soil phosphate was much more rapid than was the velocity of solution of Fe- and Al-hydroxides, which cause re-precipitation. Extraction of a soil with 0.05 *N* HNO_3 gave the following result:

	P_2O_5	*$Fe_2O_3 + Al_2O_3$*
After 10 minutes, mgm. per 100 cc.	7.39	63.0
After 24 hours, mgm. per 100 cc.	4.56	221.0

[6] In recent investigations of Mitchell (27) $KHSO_4$ has also been found very suitable for extraction of soil phosphate.

calomel cell and a Leeds and Northrup pH indicator. Stocks of $KHSO_4$ from different sources have varied somewhat in acidity at the same concentrations.

The soil extracts with $KHSO_4$ were easily filtered because of the flocculating effect of this solvent. Also, organic matter, iron, and silica were not dissolved in sufficient amounts to interfere with the colorimetric determinations.

$$x\ KHSO_4 \rightleftharpoons x\ K^+ + x\ HSO_4^-,$$
$$x\ HSO_4^- \rightleftharpoons x\ H^+ + (x - y)\ HSO_4^-,\ (x > y)$$

TABLE 1

The hydrogen-ion concentration of $KHSO_4$-solutions of different concentrations

$KHSO_4$ IN 1,000 CC.	MOLAR CONCENTRATION	pH OF THE SOLUTION
gm.		
13.617	0.100	1.40
2.723	0.020	1.91
2.383	0.018	1.95
2.092	0.015	2.00
1.362	0.010	2.14
0.800	0.006	2.32
0.650	0.005	2.40
0.500	0.004	2.50
0.250	0.002	2.74
0.136	0.001	3.06
0.0136	0.0001	4.08

TABLE 2

The influence of varying amounts of soil to 200 cc. 0.01 M $KHSO_4$ upon the amounts of phosphate dissolved from two soils

WELLAND SOIL (SURFACE; pH 4.61)				MARKDALE SOIL (SURFACE; pH 7.63)			
Soil used	P in extract	P in 1,000 gm. soil	pH of extract	Soil used	P in extract	P in 1,000 gm. soil	pH of extract
gm.	*mgm.*	*mgm.*		*gm.*	*mgm.*	*mgm.*	
2	0.210	105	2.31	2	0.044	22	2.46
4	0.325	81	2.32	4	0.055	14	2.56
10	0.725	73	2.49	10	0.089	9	3.09
50	0.170	3	3.28	50	0.029	0.6	5.48

Various amounts of two soils were shaken 5 minutes with 200 cc. 0.01 *M* $KHSO_4$ and filtered rapidly at once (suction) through a Buchner funnel with an inside diameter of 9½ cm. (# 42 acid-washed Whatman filter paper).[7] The pH of the solvent was 2.30 (the disagreement in this respect with the results in table 2 is due to the fact that the salt used was of another preparation).

[7] In all experiments presented here, this size of funnel is used in order that the filtering can be as rapid as possible. Also, Whatman # 42 acid-washed filter paper only is used. (The filter papers were always tested before use.)

It is significant that with the first three amounts of soil, the pH values of the solvent after the extraction have not gone above 3.09[8] and the amounts of phosphate extracted are proportional with the amounts of soil used. By using 50 gm. of soil to 200 cc. of this solvent, the acidity of the solvent is

TABLE 3

The influence of removal of dissolved organic matter and silica upon the phosphate content in 1:200 0.01 M $KHSO_4$-extracts of two soils

SOIL	TIME OF SHAKING	P IN 1,000 GM. SOIL	
		Organic matter and silica removed	Organic matter and silica *not* removed
	minutes	*mgm.*	*mgm.*
Markdale surface soil	15	27	22
	30	33	27
Markdale subsoil*	15	29	20
	30	32	23
Welland No. 2 surface soil	15	149	129
	30	204	174

* pH 7.51.

TABLE 4

*Solubility of soil phosphate in two subsoils at different acidities**

SOIL	pH OF SOLVENT BEFORE EXTRACTION	pH OF SOLVENT AFTER EXTRACTION	P DISSOLVED PER 1,000 GM. SOIL
			mgm.
Markdale subsoil (pH 7.51)	2.18	2.22	23
	2.00	2.04	30
	1.80	1.81	32
	1.44	1.46	51
Welland subsoil (pH 5.22)	2.18	2.18	22
	2.00	2.04	25
	1.80	1.84	32
	1.44	1.45	35

* Experiments with the Markdale soil with extractions with increasing amounts of HCl and HNO_3 showed the same trend.

decreased to a range where re-precipitation of dissolved phosphate takes place, and the phosphate content of the extracts is consequently very low.

In the evaporated aliquots organic matter was destroyed by ignition, after

[8] It is also important to note that the pH of 3.09 is obtained in extraction of the Markdale soil, which is a highly calcareous soil.

TABLE 5

The influence of time of shaking upon the amounts of phosphate extracted from soils in 1:200 extracts with $KHSO_4$ at pH 2.00

SOIL	TREATMENT IN THE FIELD	TIME OF SHAKING	P IN 1,000 GM. SOIL	pH AFTER EXTRACTION
		minutes	*mgm.*	
Markdale surface	Summer fallow*	5	23	2.01
		15	26	2.02
		30	29	2.02
Markdale subsoil		5	24	2.05
		15	28	2.07
		30	31	2.10
Welland permanent fertility plots:				
No. 1 surface	400 lbs. acid phosphate per acre	5	39	2.00
		15	47	2.00
No. 2 surface	1,000 lbs. rock phosphate + 500 lbs. S per acre	5	180	2.00
		15	186	1.99
		30	224	2.00
subsoil	*	5	23	2.05
		15	25	2.03
		30	25	2.05
No. 3 surface	2 tons lime per acre	5	27	2.02
		15	27	2.03
No. 4 surface	2 tons lime + 400 lbs. acid phosphate per acre	5	38	2.00
		15	48	2.01
No. 5 surface	2 tons lime + 1,000 lbs. rock phosphate per acre	5	248	2.01
		15	260	2.00
No. 6 surface	2 tons lime + 600 lbs. basic slag per acre	5	69	2.03
		15	69	2.02
No. 7 surface	2 tons lime + 100 lbs. sulfate of ammonia + 400 lbs. acid phosphate + 100 lbs. muriate of potash per acre	5	41	2.02
		15	45	2.02
No. 11 surface	No treatment	5	24	2.01
		15	33	2.01

* The aforementioned Markdale and Welland soils.

evaporation with $Mg(NO_3)_2$, and silica was removed by centrifugation after acid dehydration.

To determine the influence of the acidity of the solvent upon the solubility of difficultly soluble soil phosphate, 2-gm. portions of each of the subsoils from Markdale and Welland were shaken 15 minutes with 400 cc. of $KHSO_4$ solutions of different acidities.

TABLE 6

Readily soluble phosphates extracted from phosphate treated soil samples

SOIL IN 200 CC. KH_2PO_4 SOLUTION	P REMOVED BY SOIL FROM KH_2PO_4 SOLUTION	AMOUNT OF P EXTRACTED FROM SOIL BY $KHSO_4$ METHOD*		INCREASE OF DISSOLVED P DUE TO THE P REMOVED FROM KH_2PO_4 SOLUTION	PER CENT OF THE P REMOVED BY SOIL RECOVERED IN $KHSO_4$ EXTRACT
		Phosphate treated soil	Untreated soil		
gm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
Welland soil:					
Surface soil					
100	22.6	21.0	18.0	3.0	13
50	20.3	14.7	9.0	5.7	28
10	7.0	5.4	1.8	3.6	51
Subsoil					
100	22.4	10.7	3.0	7.7	34
50	19.6	11.0	1.5	9.5	48
10	7.7	3.4	0.3	3.1	40
Markdale soil:					
Surface soil					
100	22.6	10.7	2.3	8.4	37
50	19.3	10.8	1.2	9.6	50
10	10.4	2.8	0.2	2.6	25
Subsoil					
100	21.4	10.5	2.4	8.1	38
50	15.5	9.3	1.2	8.1	54
10	9.2	3.0	0.2	2.8	30

* Comparisons between the Truog and the $KHSO_4$ methods are given in tables 7, 8, and 9.

Then the $KHSO_4$ solution at a pH of 2.00 was chosen for a study of the influence of time upon the rate of dissolution of soil phosphate from soils well known in so far as their phosphate treatment and response to phosphatic fertilizers was concerned. Two grams of soil was shaken 5 minutes with 400 cc. of $KHSO_4$ and filtered at once. The $KHSO_4$ stock used for the measurements reported in table 1 was used here and in all the following experiments.

As the amounts of the difficultly soluble soil phosphates dissolved do not increase appreciably as a result of prolonged shaking, 5 minutes of shaking and 2 gm. of soil to 400 cc. $KHSO_4$ at a pH of 2.00 were chosen for further experi-

TABLE 7

Readily soluble phosphate in samples of surface and subsoils from Welland fertility plots, as determined by the $KHSO_4$ and the Truog methods

PLOT NUMBER	pH OF SUSPENSION (1:1)	$KHSO_4$ METHOD		TRUOG METHOD	
		P per 1,000 gm. soil	pH of extracts	P per 1,000 gm. soil	pH of extracts
		mgm.		*mgm.*	
Surface soils					
1	5.67	39	2.02	22	3.15
2	4.61	180	2.00	92	3.12
3	6.11	24	2.02	16	3.05
4	5.58	38	2.00	26	3.08
5	6.30	248	2.01	207	3.10
6	6.92	69	2.03	..	....
7	6.15	41	2.02	..	....
10*	5.54	32	2.01	..	....
11	5.25	24	2.01	..	....
Subsoils					
1	6.00	116	2.05	76	3.15
2	5.22	23	2.02	11	3.01
3	5.83	59	2.02	41	3.03
4	5.46	57	2.02	43	3.07
5	5.63	80	2.02	52	3.20
6	6.06	110	2.03	..	....
7	5.53	47	2.01	..	....
10*	5.25	12	2.03	..	....
11	5.11	9	2.03	..	....

* Plot 10 has received 2 tons lime + 100 pounds sulfate of ammonia + 400 pounds acid phosphate per acre. For explanation of the treatment of the other plots, see table 5.

TABLE 8

Readily soluble phosphate in the profile of the Guelph sandy loam as determined by the $KHSO_4$ method and the Truog method

HORIZON	pH*	TOTAL CONTENT OF P PER 1,000 GM. SOIL	$KHSO_4$ METHOD		TRUOG METHOD	
			P per 1,000 gm. soil	pH of extract	P per 1,000 gm. soil	pH of extract
		mgm.	*mgm.*		*mgm.*	
A_1	7.94	1,267	38	2.03	24	3.24
A_2	7.68	1,049	18	2.04	11	3.15
A_3	7.68	1,092	47	2.02	25	3.10
B_1	7.66	1,136	69	2.02	38	3.42
B_2	8.27	1,223	122	2.06	39	5.15
C_2	8.01	786	56	2.33	..	....
..	....	.....	73†	2.02†	7	6.54

* Analysis after Ruhnke (31).

† By adjustment of the solvent with 0.700 gm. $KHSO_4$.

mentation. In the following for convenience this extraction is called the "$KHSO_4$-method."

In order to get an idea as to the dissolving capacity under these experimental conditions, the method was tried on samples from a fixation experiment with the Welland and Markdale soils. To 200 cc. of a KH_2PO_4-solution containing

TABLE 9

Readily soluble phosphate in a fertile garden soil as determined by the $KHSO_4$ and the Truog methods*

	P PER 1,000 GM. SOIL	pH OF EXTRACTS
	mgm.	
Truog method	290	5.27
$KHSO_4$ method	450	2.05

* Total phosphorus = 1,200 mgm. P in 1,000 gm. soil. pH 8.18.

TABLE 10

Soil phosphate dissolved in $KHSO_4$ solvents at the pH values of 3.00 and 3.80

NUMBER OF PLOT	$KHSO_4$ AT A pH OF 3.00		$KHSO_4$ AT A pH OF 3.80	
	P per 1,000 gm. soil	pH of extract	P per 1,000 gm. soil	pH of extract
	mgm.		*mgm.*	
Surface soils				
1	12.5	3.20	9	4.45
2	43	3.12	16.5	4.10
3	8.5	3.27	6	5.07
4	14.5	3.20	8.5	4.91
5	88	3.22	16	4.58
Subsoils				
1	41	3.17	14.5	4.42
2	3.5	3.13	2	4.47
3	22	3.16	9	4.58
4	20	3.16	8.5	4.50
5	23	3.17	10	4.58
Garden soil surface	206	4.98	74	6.01

25 mgm. P 100, 50, and 10 gm. of soil were added and left standing, with frequent shaking, for 17 hours, following which the phosphate content in the solution was determined. Then the soil was sucked as dry as possible on a large Buchner funnel, air-dried, and extracted by the $KHSO_4$-method as described. Samples of the same soils without phosphate treatment were also extracted.

$KHSO_4$ solvents of other concentrations have been used with five samples

from the Welland plots and the garden soil (2 gm. soil to 400 cc. $KHSO_4$, 5 minutes of shaking, see table 10, and fig. 1).

Surface soils 1, 2, and 3 from Welland were shaken 5 minutes with CO_2-free distilled water (soil:solvent = 1:100). No dissolved phosphate could be

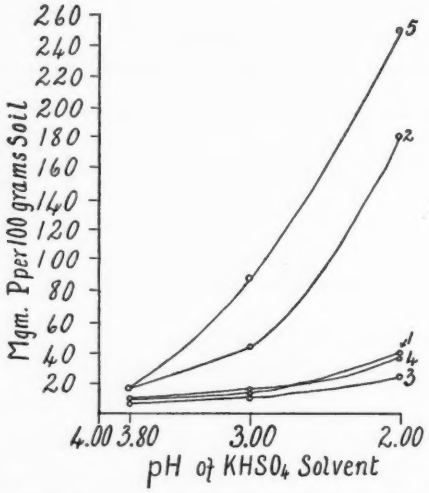

FIG. 1. SOIL PHOSPHATE DISSOLVED IN $KHSO_4$ SOLVENTS OF DIFFERENT ACIDITIES
Samples from Welland Plots

detected. By repeating water extraction in the same way after having dehydrated the soils 36 hours at 105°C. the following results were obtained:

SOIL NUMBER	P PER 1,000 GM. SOIL	pH OF EXTRACT
	mgm.	
1	8	5.41
2	9	5.49
3	Negligible	5.59

The garden soil (see table 9), in which the amount of readily soluble phosphate is rather high, was leached with several solvents, and the phosphate content in the subsequent leachates determined. Ten grams of the fresh soil was spread in a thin layer on a Buchner funnel (9.5 cm. in diameter, Whatman #42, acid-washed filter paper), and 100-cc. portions of the different solvents were poured on in portions of 25 cc. (see table 11 and fig. 2).

The leachings with the $KHSO_4$ (pH 2.00) were continued until all of the more readily soluble phosphate was removed (see table 12).

Figure 3 shows that 10 leachings with $KHSO_4$ (at a pH of 2.00) have re-

TABLE 11

The influence of the acidity of the solvent in dissolving soil phosphate by leaching of a fresh fertile garden soil

NUMBER OF LEACHATE	DISTILLED WATER (FREE FROM CO_2)		0.002 *N* H_2SO_4 BUFFERED TO pH 3.00 (SOLVENT TO TRUOG METHOD)		$KHSO_4$ AT pH 2.00 (SOLVENT TO $KHSO_4$ METHOD)		0.1 *N* KCl + HCl; pH 1.50	
	P in 100 cc. fresh leachate	pH of leachate	P in 100 cc. leachate	pH of leachate	P in 100 cc. leachate	pH of leachate	P in 100 cc. leachate	pH of leachate
	mgm.		*mgm.*		*mgm.*		*mgm.*	
1	0.132	6.78	0.250	6.58	0.800	2.81	1.150	1.97
2	0.050	6.05	0.225	7.00				

ERRATUM

Studies on readily soluble phosphate in soils: I.

By H. W. Lohse and G. N. Ruhnke, SOIL SCIENCE, Vol. XXXV

Page 447: The ordinate of figure 2, *Mgm. P in 100 cc. Leachate,* should read *Mgm. P Leached.*

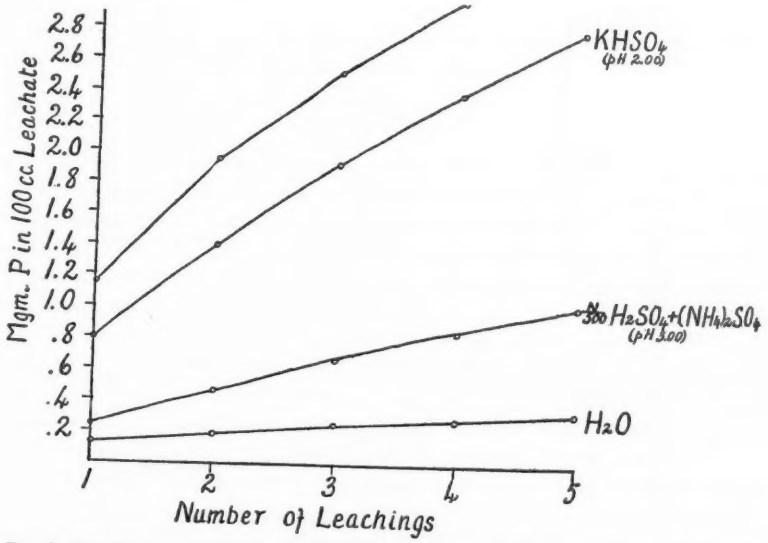

FIG. 2. THE INFLUENCE OF THE ACIDITY OF THE SOLVENT IN DISSOLVING SOIL PHOSPHATE BY LEACHING A FRESH FERTILE GARDEN SOIL

TABLE 12

Leaching of a fresh fertile garden soil with $KHSO_4$ at a pH of 2.00*

NUMBER OF LEACHING	P IN 100 CC. OF LEACHATE	pH OF EXTRACTS	NUMBER OF LEACHING	P IN 100 CC. OF LEACHATE	pH OF EXTRACTS
	mgm.			*mgm.*	
1	.800	2.81	17	....	....
2	.633	2.51	18	.130	2.30
3	.500	2.37	19	....	....
4	.450	2.37	20	.092	2.25
5	.400	2.31	21	....	....
6	.270	2.37†	22	.090	2.26
7	....	....	23	....	....
8	.240	2.30	24	.078	2.26
9	....	....	25	....	....
10	.180	2.22	26	.094	2.23
11	....	....	27	....	....
12	.129	2.19	28	.080	2.21
13	....	....	29	....	....
14	.108	2.20	30	.063	2.22
15	....	....†	31	....	....
16	.130	2.26	32	.058	2.22

* Fresh soil — per cent moisture = 16.32 (dried 24 hours at 105°C.). Air-dried soil — per cent moisture = 2.51.

† Interruption.

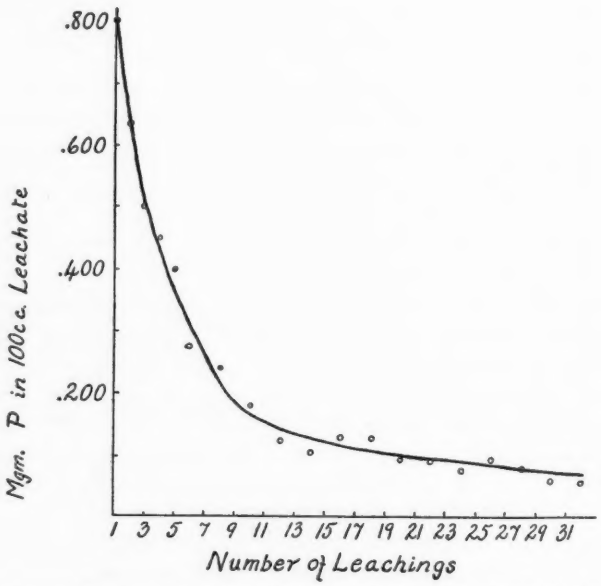

FIG. 3. CONTINUED LEACHING OF A FRESH FERTILE GARDEN SOIL WITH $KHSO_4$ AT A pH VALUE OF 2.00

moved about all of the readily soluble soil phosphate as extracted at this acidity. Calculated on air-dry basis, the leachates from the 10 leachings contained 457 mgm. of P per 1,000 gm. of soil. Five minutes of shaking the air-dry soil with the same solvent removed 450 mgm. of P per 1,000 gm. of soil (table 9). Thus, it is seen that 5 minutes of shaking removes practically all of the more readily soluble phosphate.

The $KHSO_4$ solvent, pH 2.00, has also been tried on phosphatic materials. The amounts of P dissolved from such materials are far greater than the amounts found in any soil. In table 13 are given the results from extractions of a rock phosphate, which has been used in many experiments in this institution. One gram finely ground rock phosphate (100 mesh) was shaken for

TABLE 13

The dissolution rate of a rock phosphate in a $KHSO_4$ solution at a pH of 2.00

TIME OF SHAKING	pH OF EXTRACT	P DISSOLVED FROM 1 GM. ROCK PHOSPHATE	TOTAL CONTENT OF P IN 1 GM. ROCK PHOSPHATE	P DISSOLVED IN THE $KHSO_4$ SOLUTION
minutes		*mgm.*	*mgm.*	*per cent*
1	2.42	40.2	135.5	30
3	2.50	40.5	135.5	30
5	2.57	44.4	135.5	33
15	2.71	50.3	135.5	39
30	2.71	53.6	135.5	39

varying periods of time with 400 cc. $KHSO_4$ at a pH of 2.00. P was determined after the Fiske-Subbarrow method (10).

DISCUSSION

It seems logical to assume that any acid solvent to be used in extraction of readily soluble phosphate from soils must possess an acidity of such a strength that the bulk of readily soluble phosphate in the soil sample is extracted; that the acid does not break up difficultly soluble soil phosphates appreciably; that the final acidity of the solvent is at a pH value such that re-precipitation of dissolved phosphate is prevented.

Readily soluble soil phosphate is defined as the amount of phosphate extracted, expressed as milligram P per 1,000 gm. of soil, by shaking 2 gm. of soil (20 mesh) for 5 minutes with a $KHSO_4$ solution of such a strength that the acidity of the filtered extracts is approximately 2.00. A solvent of this acidity has, for Ontario soils thus far tested, proved to fulfil the requirements mentioned.

Acid extraction of soil phosphate may be considered to be of the same value for estimating the solubility of phosphate as acid extractions of phosphatic fertilizer materials. A preliminary study on the solubility of synthetic phosphates and phosphatic fertilizer materials in a dilute acid solvent (pH 1.48) supports this assumption (20).

The data presented in table 10 and figure 1 show that the content of readily soluble soil phosphate is most clearly exposed in the series of extractions where the strongest solvent is used.

Plots 2 and 5, where the largest contents of readily soluble phosphate are found, have been treated with a ground rock phosphate, which, fixation experiments with phosphates have shown, is too difficultly soluble to be appreciably converted into the even more difficultly soluble soil phosphates (20). In the samples from plots 1, 3, and 4, in which the soil phosphate appears to be of a very difficultly soluble nature, the increased acidity of the solvents has not caused nearly so large an increase of dissolved phosphate as in the samples from plots 2 and 5.

Extractions of the samples from the Guelph profile (table 8) and from the fixation experiment (table 6) also reveal that the more difficultly soluble soil phosphates do not dissolve appreciably when the solvent is used at a pH of 2. It is interesting to note in this connection that Schloesing and de'Sigmond (34) have also found that difficultly soluble soil phosphates do not readily dissolve in this range of acidity. Studies on leached podzol profiles and very infertile soils from Ontario, have also proved this (21). The results from the Guelph profile (table 8) show that the solvent used in the Truog method is too weak to take care of the readily soluble bases in the samples from the B_2 and C horizons.

From table 2 it is seen that it is very important to extract readily soluble phosphate from soils at a pH value of the solvent, such that re-precipitation of the dissolved phosphate is prevented. This experiment also reveals that the amounts of phosphate extracted are proportional to the amounts of soil used when the pH value of the extracts is below approximately 3. In those cases where the pH values of the extracts are appreciably above 3, the amounts of phosphate extracted decrease considerably. The most plausible explanation is that some of the phosphate at first dissolved has been re-precipitated, as an excess of bases—and especially those of iron and aluminum—may cause almost complete precipitation of the phosphate ion above a pH of 3 (11). It must here be borne in mind that iron and aluminum do not need to enter into solution[9] in order to precipitate phosphate from a solution, as colloidal iron and aluminum hydroxides readily combine with phosphate in solution (11, 14). It is noteworthy that by acid extraction of the Welland soil (No. 2) the final pH of the extract was 3.28, only 3 mgm. of P per 1,000 gm. of soil was extracted, whereas by 1:500 water extraction, 9 mgm. P per 1,000 gm. of soil was extracted. The leaching experiments (table 11) in which a very thin layer of soil has been leached show that the acidity of the solvent greatly influences the amounts of phosphate leached from the soil.

The experiment with continuous leaching with $KHSO_4$ at a pH of 2 (table 12 and fig. 3) shows that 10 leachings remove practically all of the readily

[9] Practically no iron could be detected in the filtrates.

soluble phosphate, as determined at this acidity. Five minutes of shaking with the same solvent removes practically the same amount (table 9). All the experiments with solvents of different degrees of acidity reveal that the acidity of the solvent largely determines the amounts of phosphate extracted from soils containing much readily soluble phosphate, whereas from soils containing difficultly soluble phosphate the amounts of extracted phosphate do not increase to nearly the same extent. The $KHSO_4$ solvent, at a pH of 2.00, has, therefore, been chosen for the extraction of readily soluble soil phosphate from Ontario soils, because it is strong enough to expose the content of readily soluble phosphate in a soil sample, and to maintain a pH of approximately 2.00 in extraction of soil samples free from an appreciable amount of free carbonates. Besides this, difficultly soluble soil phosphates do not dissolve appreciably in this solvent. [See also Lohse and Ruhnke (21).]

Samples from surface and subsurface horizons in cultivated soils and A and B horizons in virgin soils very rarely cause an appreciable rise of the pH of the $KHSO_4$ solvent (pH 2.00).[10] But in extracting phosphate from samples of calcareous C horizons in virgin profiles and the lower horizons of agricultural soils, which often contain a considerable amount of free carbonates, the acidity of the $KHSO_4$ solvent often decreased considerably. When acid is being added to such a soil sample the primary reaction mainly taking place is

$$CaCO_3 + 2H^+ = Ca^{++} + \overbrace{H_2CO_3}^{H_2O + CO_2}$$

If the acid has only sufficient acidity to react with the carbonates present, practically no action of the acid on the phosphates can be expected to take place, and in some cases no phosphate has been extracted from such samples if the acidity of the solvent has not been corrected so as to yield an extract of pH approximately 2 (21).

Table 14 reveals the results of extraction of the C horizons of a Brown Forest soil—Kent profile (20, 21). The content of readily soluble phosphate in soil samples from C horizons containing free carbonates is usually relatively high (21).

Studies on calcareous soils in Arizona have shown that calcareous soils may contain a relatively large amount of phosphate extracted by dilute acid (Dyer and Truog methods), although they are phosphate deficient for crop growth, as a result of poor aeration (22). On soils with a high content of free carbonates in the upper layers, acid extraction of soil phosphate seems to be of limited value in determining phosphate deficiency for the crops grown there. Under humid conditions, however, where the carbonates are leached out of the upper soil horizons, extraction with dilute $KHSO_4$, as outlined in this paper, seems to give an *explicit index to the stock of readily soluble phos-*

[10] In the extraction of samples from soil types where no former experience has been obtained, acidity control of the extracts is advisable.

phate in the soil. As shown by Lohse and Ruhnke (21), such investigations should never be confined to the surface layer alone, because the subsequent layers often contain considerable amounts of readily soluble phosphate, which, no doubt, in many cases—especially in the case of deep-rooted crops—is utilized by the plants. It has been the experience in this institution that soil samples containing 30 mgm. or less of P per 1,000 gm. of soil, appear to indicate very marked phosphate deficiency. If two or three subsequent horizons are as low in extracted phosphate, the phosphate deficiency has been extremely marked. It should be mentioned that fertile soils for truck crops often contain as much as 500 to 600 mgm. of P per 1,000 gm. of soil and extremely fertile greenhouse soils run as high as about 1,000 mgm. P per 1,000 gm. of soil.

TABLE 14

Extraction of readily soluble phosphate from calcareous C-horizons from a Kent profile

HORIZON	pH*	CaO* (TOTAL)	CO_2* (TOTAL)	EXTRACTION WITH $KHSO_4$					EXTRACTION WITH HCl†	
				pH of extracts	P per 1,000 gm. soil ($KHSO_4$ solvent, pH 2.00)	Added gm. $KHSO_4$ to 400 cc. solvent, pH 2.00	pH of extract	P per 1,000 gm. soil	pH of extract	P per 1,000 gm. soil
		per cent	*per cent*		*mgm.*			*mgm.*		*mgm.*
C_1	7.77	14.32	12.16	7.11	44	2.000	2.00	270	2.04	300
C_2	7.87	10.10	8.72	6.63	11	1.200	2.03	282	2.03	295

* Analysis after Ruhnke (31).

† Buffered with KCl.

No attempt has as yet been made to settle definite limits for an adequate phosphate supply for the different crops on different soil types. Such limits can only be determined by experience obtained by future collaborative laboratory and field experiments, and generalization will always be difficult because factors such as climate, crop species, and soil management have their influence upon crop production (3). It must be said, however, that control with field results (from Ontario) has shown that phosphate extractions of soils by the $KHSO_4$ method always have given a very useful index to the fertility of the soil with respect to phosphate.

In the light of the foregoing experimental data, there seems to be little that would warrant the use of the term *available* as applied to the amounts of soil phosphate extracted by acid extraction methods. By varying the experimental conditions, the amounts of phosphate extracted will also vary; and it is quite possible so to arrange the conditions that the extracted amounts of phosphate will be of approximately the same level as the amounts found in crops. However, in order to achieve this result, it will be necessary, if acid

extraction methods are used, to use either a *narrow* soil to solvent ratio[11] or a *very weak* acid solvent. Tables 2 and 8 indicate, however, that neither of these conditions is desirable and that both may lead to fallacious results. If solvents weaker than pH 2 were used, leachings through a *very thin layer of soil* would, no doubt, be the best, as it would, in this way, be possible to control re-precipitation of dissolved phosphate. Experimentation on this point, however, has not yielded very satisfactory results.

CONCLUSIONS

Since extensive studies of typical soils and also comparison with several other methods have shown that a satisfactory index to the amount of readily soluble phosphate in soil samples is obtained by extraction with dilute $KHSO_4$ at a pH of 2.00, the following extraction method has been adopted:

Procedure for extraction of readily soluble phosphate from soils by means of dilute $KHSO_4$ at a pH of 2.00 ($KHSO_4$-method). Place 2 gm. of air-dried and sieved (20-mesh) soil in a 750-cc. Erlenmeyer flask, add 400 cc. of a $KHSO_4$ solution at a pH of 2.00 and shake 5 minutes. (Hand shaking is sufficient.) Filter at once, by using suction, through a high quality filter paper on a Buchner funnel with an inside diameter of 9½ cm. Rapid filtering—only a few minutes—is necessary. The supernatant liquid only should be poured on the filter. Discard the first 50 cc. of the filtrate, and if the filtrate is still not clear, discard the filtrate until a clear filtrate is obtained. In rare cases, a double layer of filter paper may be necessary. It is advisable to wash the filter paper by sucking about 50 cc. of water through it in two or three portions before filtering the extract. For the colorimetric determination of the phosphate, the Deniges method, as worked out by Truog and Meyer (37) is suitable, even though other colorimetric methods can also be employed (12, 13). In the most ordinary cases, 50 cc. of the filtrate can be used directly for the colorimetric determination without dilution or concentration. As the $KHSO_4$ solvent possesses a relatively high acidity, adjustment for this is most easily done in preparing the ammonium-molybdate-sulfuric acid solution if the Truog and Meyer method is used. It can also be done by means of dilute NH_4OH. According to Chapman (4), it is desirable to add the crude extract *to* the acid molybdate mixture so as to avoid formation of persistent silico-molybdate if dissolved silica should be in the solution. It is most convenient to mix the molybdate solution and the extract in 100-cc. Erlenmeyer flasks. If As^V or Fe^{III} are present, they must be reduced by means of a reductor or H_2S (4).

Only high-grade, previously tested filter papers (4, 36, 37) should be used. Suitable filter papers for this purpose are *Whatman No. 42, C. Schleicher and Schull, No. 589, White Ribbon, and Berzelius No. O. B.* It is also important to run a blank determination every time new reagents are put into use and the extraction should be run in *duplicate.*

When dealing with calcareous soil samples, the pH of the extracts should always be controlled. If the pH of the extract has changed materially from 2.00, adjustment of the $KHSO_4$ solvent with solid $KHSO_4$ should be made. How this adjustment is made is shown by the following typical example:

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[11] When dissolved phosphate is to be determined directly in the crude extracts, a wide soil to solvent ratio is also preferable, because dissolved silica, organic matter, iron, etc., which, in appreciable concentrations interfere with the colorimetric determinations, thereby are diluted.

TABLE 5

The influence of time of shaking upon the amounts of phosphate extracted from soils in 1:200 extracts with $KHSO_4$ at pH 2.00

SOIL	TREATMENT IN THE FIELD	TIME OF SHAKING	P IN 1,000 GM. SOIL	pH AFTER EXTRACTION
		minutes	*mgm.*	
Markdale surface	Summer fallow*	5	23	2.01
		15	26	2.02
		30	29	2.02
Markdale subsoil		5	24	2.05
		15	28	2.07
		30	31	2.10
Welland permanent fertility plots:				
No. 1 surface	400 lbs. acid phosphate per acre	5	39	2.00
		15	47	2.00
No. 2 surface	1,000 lbs. rock phosphate + 500 lbs. S per acre	5	180	2.00
		15	186	1.99
		30	224	2.00
subsoil	*	5	23	2.05
		15	25	2.03
		30	25	2.05
No. 3 surface	2 tons lime per acre	5	27	2.02
		15	27	2.03
No. 4 surface	2 tons lime + 400 lbs. acid phosphate per acre	5	38	2.00
		15	48	2.01
No. 5 surface	2 tons lime + 1,000 lbs. rock phosphate per acre	5	248	2.01
		15	260	2.00
No. 6 surface	2 tons lime + 600 lbs. basic slag per acre	5	69	2.03
		15	69	2.02
No. 7 surface	2 tons lime + 100 lbs. sulfate of ammonia + 400 lbs. acid phosphate + 100 lbs. muriate of potash per acre	5	41	2.02
		15	45	2.02
No. 11 surface	No treatment	5	24	2.01
		15	33	2.01

* The aforementioned Markdale and Welland soils.

evaporation with $Mg(NO_3)_2$, and silica was removed by centrifugation after acid dehydration.

To determine the influence of the acidity of the solvent upon the solubility of difficultly soluble soil phosphate, 2-gm. portions of each of the subsoils from Markdale and Welland were shaken 15 minutes with 400 cc. of $KHSO_4$ solutions of different acidities.

TABLE 6

Readily soluble phosphates extracted from phosphate treated soil samples

SOIL IN 200 CC. KH_2PO_4 SOLUTION	P REMOVED BY SOIL FROM KH_2PO_4 SOLUTION	AMOUNT OF P EXTRACTED FROM SOIL BY $KHSO_4$ METHOD*		INCREASE OF DISSOLVED P DUE TO THE P REMOVED FROM KH_2PO_4 SOLUTION	PER CENT OF THE P REMOVED BY SOIL RECOVERED IN $KHSO_4$ EXTRACT
		Phosphate treated soil	Untreated soil		
gm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
Wellgnd soil:					
Surface soil					
100	22.6	21.0	18.0	3.0	13
50	20.3	14.7	9.0	5.7	28
10	7.0	5.4	1.8	3.6	51
Subsoil					
100	22.4	10.7	3.0	7.7	34
50	19.6	11.0	1.5	9.5	48
10	7.7	3.4	0.3	3.1	40
Markdale soil:					
Surface soil					
100	22.6	10.7	2.3	8.4	37
50	19.3	10.8	1.2	9.6	50
10	10.4	2.8	0.2	2.6	25
Subsoil					
100	21.4	10.5	2.4	8.1	38
50	15.5	9.3	1.2	8.1	54
10	9.2	3.0	0.2	2.8	30

* Comparisons between the Truog and the $KHSO_4$ methods are given in tables 7, 8, and 9.

Then the $KHSO_4$ solution at a pH of 2.00 was chosen for a study of the influence of time upon the rate of dissolution of soil phosphate from soils well known in so far as their phosphate treatment and response to phosphatic fertilizers was concerned. Two grams of soil was shaken 5 minutes with 400 cc. of $KHSO_4$ and filtered at once. The $KHSO_4$ stock used for the measurements reported in table 1 was used here and in all the following experiments.

As the amounts of the difficultly soluble soil phosphates dissolved do not increase appreciably as a result of prolonged shaking, 5 minutes of shaking and 2 gm. of soil to 400 cc. $KHSO_4$ at a pH of 2.00 were chosen for further experi-

TABLE 7

Readily soluble phosphate in samples of surface and subsoils from Welland fertility plots, as determined by the $KHSO_4$ and the Truog methods

PLOT NUMBER	pH OF SUSPENSION (1:1)	$KHSO_4$ METHOD		TRUOG METHOD	
		P per 1,000 gm. soil	pH of extracts	P per 1,000 gm. soil	pH of extracts
		mgm.		*mgm.*	
Surface soils					
1	5.67	39	2.02	22	3.15
2	4.61	180	2.00	92	3.12
3	6.11	24	2.02	16	3.05
4	5.58	38	2.00	26	3.08
5	6.30	248	2.01	207	3.10
6	6.92	69	2.03	..	....
7	6.15	41	2.02	..	....
10*	5.54	32	2.01	..	....
11	5.25	24	2.01	..	....
Subsoils					
1	6.00	116	2.05	76	3.15
2	5.22	23	2.02	11	3.01
3	5.83	59	2.02	41	3.03
4	5.46	57	2.02	43	3.07
5	5.63	80	2.02	52	3.20
6	6.06	110	2.03	..	....
7	5.53	47	2.01	..	....
10*	5.25	12	2.03	..	....
11	5.11	9	2.03	..	....

* Plot 10 has received 2 tons lime + 100 pounds sulfate of ammonia + 400 pounds acid phosphate per acre. For explanation of the treatment of the other plots, see table 5.

TABLE 8

Readily soluble phosphate in the profile of the Guelph sandy loam as determined by the $KHSO_4$ method and the Truog method

HORIZON	pH*	TOTAL CONTENT OF P PER 1,000 GM. SOIL	$KHSO_4$ METHOD		TRUOG METHOD	
			P per 1,000 gm. soil	pH of extract	P per 1,000 gm. soil	pH of extract
		mgm.	*mgm.*		*mgm.*	
A_1	7.94	1,267	38	2.03	24	3.24
A_2	7.68	1,049	18	2.04	11	3.15
A_3	7.68	1,092	47	2.02	25	3.10
B_1	7.66	1,136	69	2.02	38	3.42
B_2	8.27	1,223	122	2.06	39	5.15
C_2	8.01	786	56	2.33	..	....
..	....	.....	73†	2.02†	7	6.54

* Analysis after Ruhnke (31).

† By adjustment of the solvent with 0.700 gm. $KHSO_4$.

mentation. In the following for convenience this extraction is called the "$KHSO_4$-method."

In order to get an idea as to the dissolving capacity under these experimental conditions, the method was tried on samples from a fixation experiment with the Welland and Markdale soils. To 200 cc. of a KH_2PO_4-solution containing

TABLE 9

Readily soluble phosphate in a fertile garden soil as determined by the $KHSO_4$ and the Truog methods*

	P per 1,000 gm. soil	pH of extracts
	mgm.	
Truog method	290	5.27
$KHSO_4$ method	450	2.05

* Total phosphorus = 1,200 mgm. P in 1,000 gm. soil. pH 8.18.

TABLE 10

Soil phosphate dissolved in $KHSO_4$ solvents at the pH values of 3.00 and 3.80

Number of plot	$KHSO_4$ at a pH of 3.00		$KHSO_4$ at a pH of 3.80	
	P per 1,000 gm. soil	pH of extract	P per 1,000 gm. soil	pH of extract
	mgm.		*mgm.*	
Surface soils				
1	12.5	3.20	9	4.45
2	43	3.12	16.5	4.10
3	8.5	3.27	6	5.07
4	14.5	3.20	8.5	4.91
5	88	3.22	16	4.58
Subsoils				
1	41	3.17	14.5	4.42
2	3.5	3.13	2	4.47
3	22	3.16	9	4.58
4	20	3.16	8.5	4.50
5	23	3.17	10	4.58
Garden soil surface	206	4.98	74	6.01

25 mgm. P 100, 50, and 10 gm. of soil were added and left standing, with frequent shaking, for 17 hours, following which the phosphate content in the solution was determined. Then the soil was sucked as dry as possible on a large Buchner funnel, air-dried, and extracted by the $KHSO_4$-method as described. Samples of the same soils without phosphate treatment were also extracted.

$KHSO_4$ solvents of other concentrations have been used with five samples

from the Welland plots and the garden soil (2 gm. soil to 400 cc. $KHSO_4$, 5 minutes of shaking, see table 10, and fig. 1).

Surface soils 1, 2, and 3 from Welland were shaken 5 minutes with CO_2-free distilled water (soil:solvent = 1:100). No dissolved phosphate could be

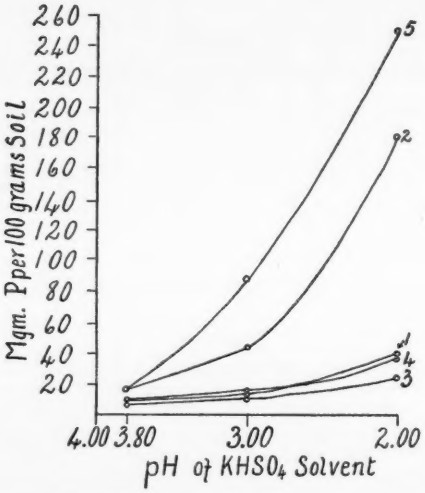

FIG. 1. SOIL PHOSPHATE DISSOLVED IN $KHSO_4$ SOLVENTS OF DIFFERENT ACIDITIES
Samples from Welland Plots

detected. By repeating water extraction in the same way after having dehydrated the soils 36 hours at 105°C. the following results were obtained:

SOIL NUMBER	P PER 1,000 GM. SOIL	pH OF EXTRACT
	mgm.	
1	8	5.41
2	9	5.49
3	Negligible	5.59

The garden soil (see table 9), in which the amount of readily soluble phosphate is rather high, was leached with several solvents, and the phosphate content in the subsequent leachates determined. Ten grams of the fresh soil was spread in a thin layer on a Buchner funnel (9.5 cm. in diameter, Whatman #42, acid-washed filter paper), and 100-cc. portions of the different solvents were poured on in portions of 25 cc. (see table 11 and fig. 2).

The leachings with the $KHSO_4$ (pH 2.00) were continued until all of the more readily soluble phosphate was removed (see table 12).

Figure 3 shows that 10 leachings with $KHSO_4$ (at a pH of 2.00) have re-

ERRATUM

Studies on readily soluble phosphate in soils: I.

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Page 447: The ordinate of figure 2, *Mgm. P in 100 cc. Leachate*, should read *Mgm. P Leached.*

TABLE 11

The influence of the acidity of the solvent in dissolving soil phosphate by leaching of a fresh fertile garden soil

NUMBER OF LEACHATE	DISTILLED WATER (FREE FROM CO_2)		0.002 *N* H_2SO_4 BUFFERED TO pH 3.00 (SOLVENT TO TRUOG METHOD)		$KHSO_4$ AT pH 2.00 (SOLVENT TO $KHSO_4$ METHOD)		0.1 *N* KCl + HCl; pH 1.50	
	P in 100 cc. fresh leachate	pH of leachate	P in 100 cc. leachate	pH of leachate	P in 100 cc. leachate	pH of leachate	P in 100 cc. leachate	pH of leachate
	mgm.		*mgm.*		*mgm.*		*mgm.*	
1	0.132	6.78	0.250	6.58	0.800	2.81	1.150	1.97
2	0.059	6.95	0.225	7.06	0.633	2.51	0.800	1.79
3	0.055	6.85	0.193	6.99	0.500	2.37	0.562	1.66
4	0.048	....	0.178	7.05	0.450	2.37	0.465	1.67
5	0.046	6.90	0.173	7.15	0.400	2.31	0.375	1.64
Total amount of P leached out in 5 leachings, mgm. P..	0.340		1.019		2.783		3.352	

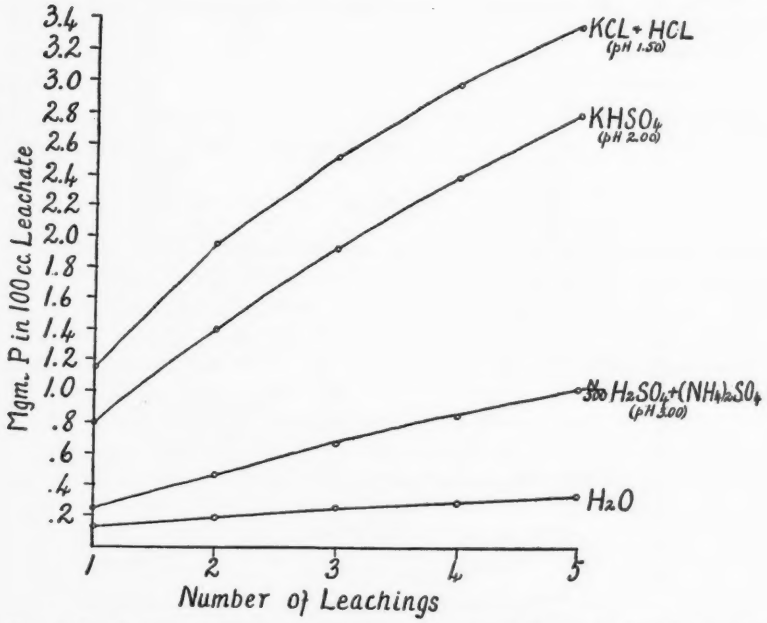

FIG. 2. THE INFLUENCE OF THE ACIDITY OF THE SOLVENT IN DISSOLVING SOIL PHOSPHATE BY LEACHING A FRESH FERTILE GARDEN SOIL

TABLE 12

Leaching of a fresh fertile garden soil with $KHSO_4$ at a pH of 2.00*

NUMBER OF LEACHING	P IN 100 CC. OF LEACHATE	pH OF EXTRACTS	NUMBER OF LEACHING	P IN 100 CC. OF LEACHATE	pH OF EXTRACTS
	mgm.			*mgm.*	
1	.800	2.81	17	....	....
2	.633	2.51	18	.130	2.30
3	.500	2.37	19	....	....
4	.450	2.37	20	.092	2.25
5	.400	2.31	21	....	....
6	.270	2.37†	22	.090	2.26
7	....	....	23	....	....
8	.240	2.30	24	.078	2.26
9	....	....	25	....	....
10	.180	2.22	26	.094	2.23
11	....	....	27	....	....
12	.129	2.19	28	.080	2.21
13	....	....	29	....	....
14	.108	2.20	30	.063	2.22
15	....	....†	31	....	....
16	.130	2.26	32	.058	2.22

* Fresh soil — per cent moisture = 16.32 (dried 24 hours at 105°C.). Air-dried soil — per cent moisture = 2.51.

† Interruption.

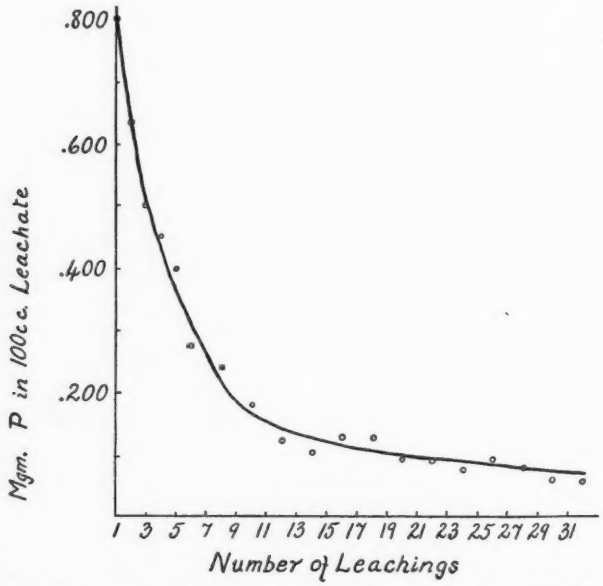

FIG. 3. CONTINUED LEACHING OF A FRESH FERTILE GARDEN SOIL WITH $KHSO_4$ AT A pH VALUE OF 2.00

moved about all of the readily soluble soil phosphate as extracted at this acidity. Calculated on air-dry basis, the leachates from the 10 leachings contained 457 mgm. of P per 1,000 gm. of soil. Five minutes of shaking the air-dry soil with the same solvent removed 450 mgm. of P per 1,000 gm. of soil (table 9). Thus, it is seen that 5 minutes of shaking removes practically all of the more readily soluble phosphate.

The $KHSO_4$ solvent, pH 2.00, has also been tried on phosphatic materials. The amounts of P dissolved from such materials are far greater than the amounts found in any soil. In table 13 are given the results from extractions of a rock phosphate, which has been used in many experiments in this institution. One gram finely ground rock phosphate (100 mesh) was shaken for

TABLE 13

The dissolution rate of a rock phosphate in a $KHSO_4$ solution at a pH of 2.00

TIME OF SHAKING	pH OF EXTRACT	P DISSOLVED FROM 1 GM. ROCK PHOSPHATE	TOTAL CONTENT OF P IN 1 GM. ROCK PHOSPHATE	P DISSOLVED IN THE $KHSO_4$ SOLUTION
minutes		*mgm.*	*mgm.*	*per cent*
1	2.42	40.2	135.5	30
3	2.50	40.5	135.5	30
5	2.57	44.4	135.5	33
15	2.71	50.3	135.5	39
30	2.71	53.6	135.5	39

varying periods of time with 400 cc. $KHSO_4$ at a pH of 2.00. P was determined after the Fiske-Subbarrow method (10).

DISCUSSION

It seems logical to assume that any acid solvent to be used in extraction of readily soluble phosphate from soils must possess an acidity of such a strength that the bulk of readily soluble phosphate in the soil sample is extracted; that the acid does not break up difficultly soluble soil phosphates appreciably; that the final acidity of the solvent is at a pH value such that re-precipitation of dissolved phosphate is prevented.

Readily soluble soil phosphate is defined as the amount of phosphate extracted, expressed as milligram P per 1,000 gm. of soil, by shaking 2 gm. of soil (20 mesh) for 5 minutes with a $KHSO_4$ solution of such a strength that the acidity of the filtered extracts is approximately 2.00. A solvent of this acidity has, for Ontario soils thus far tested, proved to fulfil the requirements mentioned.

Acid extraction of soil phosphate may be considered to be of the same value for estimating the solubility of phosphate as acid extractions of phosphatic fertilizer materials. A preliminary study on the solubility of synthetic phosphates and phosphatic fertilizer materials in a dilute acid solvent (pH 1.48) supports this assumption (20).

The data presented in table 10 and figure 1 show that the content of readily soluble soil phosphate is most clearly exposed in the series of extractions where the strongest solvent is used.

Plots 2 and 5, where the largest contents of readily soluble phosphate are found, have been treated with a ground rock phosphate, which, fixation experiments with phosphates have shown, is too difficultly soluble to be appreciably converted into the even more difficultly soluble soil phosphates (20). In the samples from plots 1, 3, and 4, in which the soil phosphate appears to be of a very difficultly soluble nature, the increased acidity of the solvents has not caused nearly so large an increase of dissolved phosphate as in the samples from plots 2 and 5.

Extractions of the samples from the Guelph profile (table 8) and from the fixation experiment (table 6) also reveal that the more difficultly soluble soil phosphates do not dissolve appreciably when the solvent is used at a pH of 2. It is interesting to note in this connection that Schloesing and de'Sigmond (34) have also found that difficultly soluble soil phosphates do not readily dissolve in this range of acidity. Studies on leached podzol profiles and very infertile soils from Ontario, have also proved this (21). The results from the Guelph profile (table 8) show that the solvent used in the Truog method is too weak to take care of the readily soluble bases in the samples from the B_2 and C horizons.

From table 2 it is seen that it is very important to extract readily soluble phosphate from soils at a pH value of the solvent, such that re-precipitation of the dissolved phosphate is prevented. This experiment also reveals that the amounts of phosphate extracted are proportional to the amounts of soil used when the pH value of the extracts is below approximately 3. In those cases where the pH values of the extracts are appreciably above 3, the amounts of phosphate extracted decrease considerably. The most plausible explanation is that some of the phosphate at first dissolved has been re-precipitated, as an excess of bases—and especially those of iron and aluminum—may cause almost complete precipitation of the phosphate ion above a pH of 3 (11). It must here be borne in mind that iron and aluminum do not need to enter into solution[9] in order to precipitate phosphate from a solution, as colloidal iron and aluminum hydroxides readily combine with phosphate in solution (11, 14). It is noteworthy that by acid extraction of the Welland soil (No. 2) the final pH of the extract was 3.28, only 3 mgm. of P per 1,000 gm. of soil was extracted, whereas by 1:500 water extraction, 9 mgm. P per 1,000 gm. of soil was extracted. The leaching experiments (table 11) in which a very thin layer of soil has been leached show that the acidity of the solvent greatly influences the amounts of phosphate leached from the soil.

The experiment with continuous leaching with $KHSO_4$ at a pH of 2 (table 12 and fig. 3) shows that 10 leachings remove practically all of the readily

[9] Practically no iron could be detected in the filtrates.

soluble phosphate, as determined at this acidity. Five minutes of shaking with the same solvent removes practically the same amount (table 9). All the experiments with solvents of different degrees of acidity reveal that the acidity of the solvent largely determines the amounts of phosphate extracted from soils containing much readily soluble phosphate, whereas from soils containing difficultly soluble phosphate the amounts of extracted phosphate do not increase to nearly the same extent. The $KHSO_4$ solvent, at a pH of 2.00, has, therefore, been chosen for the extraction of readily soluble soil phosphate from Ontario soils, because it is strong enough to expose the content of readily soluble phosphate in a soil sample, and to maintain a pH of approximately 2.00 in extraction of soil samples free from an appreciable amount of free carbonates. Besides this, difficultly soluble soil phosphates do not dissolve appreciably in this solvent. [See also Lohse and Ruhnke (21).]

Samples from surface and subsurface horizons in cultivated soils and A and B horizons in virgin soils very rarely cause an appreciable rise of the pH of the $KHSO_4$ solvent (pH 2.00).[10] But in extracting phosphate from samples of calcareous C horizons in virgin profiles and the lower horizons of agricultural soils, which often contain a considerable amount of free carbonates, the acidity of the $KHSO_4$ solvent often decreased considerably. When acid is being added to such a soil sample the primary reaction mainly taking place is

$$CaCO_3 + 2H^+ = Ca^{++} + \overbrace{H_2CO_3}^{H_2O + CO_2}$$

If the acid has only sufficient acidity to react with the carbonates present, practically no action of the acid on the phosphates can be expected to take place, and in some cases no phosphate has been extracted from such samples if the acidity of the solvent has not been corrected so as to yield an extract of pH approximately 2 (21).

Table 14 reveals the results of extraction of the C horizons of a Brown Forest soil—Kent profile (20, 21). The content of readily soluble phosphate in soil samples from C horizons containing free carbonates is usually relatively high (21).

Studies on calcareous soils in Arizona have shown that calcareous soils may contain a relatively large amount of phosphate extracted by dilute acid (Dyer and Truog methods), although they are phosphate deficient for crop growth, as a result of poor aeration (22). On soils with a high content of free carbonates in the upper layers, acid extraction of soil phosphate seems to be of limited value in determining phosphate deficiency for the crops grown there. Under humid conditions, however, where the carbonates are leached out of the upper soil horizons, extraction with dilute $KHSO_4$, as outlined in this paper, seems to give an *explicit index to the stock of readily soluble phos-*

[10] In the extraction of samples from soil types where no former experience has been obtained, acidity control of the extracts is advisable.

phate in the soil. As shown by Lohse and Ruhnke (21), such investigations should never be confined to the surface layer alone, because the subsequent layers often contain considerable amounts of readily soluble phosphate, which, no doubt, in many cases—especially in the case of deep-rooted crops—is utilized by the plants. It has been the experience in this institution that soil samples containing 30 mgm. or less of P per 1,000 gm. of soil, appear to indicate very marked phosphate deficiency. If two or three subsequent horizons are as low in extracted phosphate, the phosphate deficiency has been extremely marked. It should be mentioned that fertile soils for truck crops often contain as much as 500 to 600 mgm. of P per 1,000 gm. of soil and extremely fertile greenhouse soils run as high as about 1,000 mgm. P per 1,000 gm. of soil.

TABLE 14

Extraction of readily soluble phosphate from calcareous C-horizons from a Kent profile

Horizon	pH*	CaO* (total)	CO_2* (total)	Extraction with $KHSO_4$					Extraction with HCl†	
				pH of extracts	P per 1,000 gm. soil ($KHSO_4$ solvent, pH 2.00)	Added gm. $KHSO_4$ to 400 cc. solvent, pH 2.00	pH of extract	P per 1,000 gm. soil	pH of extract	P per 1,000 gm. soil
		per cent	*per cent*		*mgm.*			*mgm.*		*mgm.*
C_1	7.77	14.32	12.16	7.11	44	2.000	2.00	270	2.04	300
C_2	7.87	10.10	8.72	6.63	11	1.200	2.03	282	2.03	295

* Analysis after Ruhnke (31).

† Buffered with KCl.

No attempt has as yet been made to settle definite limits for an adequate phosphate supply for the different crops on different soil types. Such limits can only be determined by experience obtained by future collaborative laboratory and field experiments, and generalization will always be difficult because factors such as climate, crop species, and soil management have their influence upon crop production (3). It must be said, however, that control with field results (from Ontario) has shown that phosphate extractions of soils by the $KHSO_4$ method always have given a very useful index to the fertility of the soil with respect to phosphate.

In the light of the foregoing experimental data, there seems to be little that would warrant the use of the term *available* as applied to the amounts of soil phosphate extracted by acid extraction methods. By varying the experimental conditions, the amounts of phosphate extracted will also vary; and it is quite possible so to arrange the conditions that the extracted amounts of phosphate will be of approximately the same level as the amounts found in crops. However, in order to achieve this result, it will be necessary, if acid

extraction methods are used, to use either a *narrow* soil to solvent ratio[11] or a *very weak* acid solvent. Tables 2 and 8 indicate, however, that neither of these conditions is desirable and that both may lead to fallacious results. If solvents weaker than pH 2 were used, leachings through a *very thin layer of soil* would, no doubt, be the best, as it would, in this way, be possible to control re-precipitation of dissolved phosphate. Experimentation on this point, however, has not yielded very satisfactory results.

CONCLUSIONS

Since extensive studies of typical soils and also comparison with several other methods have shown that a satisfactory index to the amount of readily soluble phosphate in soil samples is obtained by extraction with dilute $KHSO_4$ at a pH of 2.00, the following extraction method has been adopted:

Procedure for extraction of readily soluble phosphate from soils by means of dilute $KHSO_4$ at a pH of 2.00 ($KHSO_4$-method). Place 2 gm. of air-dried and sieved (20-mesh) soil in a 750-cc. Erlenmeyer flask, add 400 cc. of a $KHSO_4$ solution at a pH of 2.00 and shake 5 minutes. (Hand shaking is sufficient.) Filter at once, by using suction, through a high quality filter paper on a Buchner funnel with an inside diameter of 9½ cm. Rapid filtering—only a few minutes—is necessary. The supernatant liquid only should be poured on the filter. Discard the first 50 cc. of the filtrate, and if the filtrate is still not clear, discard the filtrate until a clear filtrate is obtained. In rare cases, a double layer of filter paper may be necessary. It is advisable to wash the filter paper by sucking about 50 cc. of water through it in two or three portions before filtering the extract. For the colorimetric determination of the phosphate, the Deniges method, as worked out by Truog and Meyer (37) is suitable, even though other colorimetric methods can also be employed (12, 13). In the most ordinary cases, 50 cc. of the filtrate can be used directly for the colorimetric determination without dilution or concentration. As the $KHSO_4$ solvent possesses a relatively high acidity, adjustment for this is most easily done in preparing the ammonium-molybdate-sulfuric acid solution if the Truog and Meyer method is used. It can also be done by means of dilute NH_4OH. According to Chapman (4), it is desirable to add the crude extract *to* the acid molybdate mixture so as to avoid formation of persistent silico-molybdate if dissolved silica should be in the solution. It is most convenient to mix the molybdate solution and the extract in 100-cc. Erlenmeyer flasks. If As^V or Fe^{III} are present, they must be reduced by means of a reductor or H_2S (4).

Only high-grade, previously tested filter papers (4, 36, 37) should be used. Suitable filter papers for this purpose are *Whatman No. 42, C. Schleicher and Schull, No. 589, White Ribbon, and Berzelius No. O. B.* It is also important to run a blank determination every time new reagents are put into use and the extraction should be run in *duplicate.*

When dealing with calcareous soil samples, the pH of the extracts should always be controlled. If the pH of the extract has changed materially from 2.00, adjustment of the $KHSO_4$ solvent with solid $KHSO_4$ should be made. How this adjustment is made is shown by the following typical example:

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[11] When dissolved phosphate is to be determined directly in the crude extracts, a wide soil to solvent ratio is also preferable, because dissolved silica, organic matter, iron, etc., which, in appreciable concentrations interfere with the colorimetric determinations, thereby are diluted.

By extracting a calcareous soil sample from the C_2 horizon of the Kent profile (see table 14), the pH of the extract was 6.63 and only 11 mgm. P per 1,000 gm. of the soil was extracted. The concentration of the $KHSO_4$ in 400 cc. of solvent necessary to combine with the bases in the sample in order to obtain a pH of approximately 2.00 in the extract, was determined by graphical interpolation as follows: 2 gm. of soil were weighed out in each of two Erlenmeyer flasks. One and two grams, respectively, of solid $KHSO_4$ were dissolved in two 400-cc. portions of $KHSO_4$ at a pH of 2.00 and the solutions were added to the two Erlenmeyer flasks.[12] These were shaken 5 minutes and filtered as described in the foregoing. The pH values in the extracts were determined on the quinhydrone electrode, and the $KHSO_4$ concentrations and the pH values were plotted in a graph (fig. 4). By interpolation of the concentration of $KHSO_4$ in the extract necessary in order to obtain a pH of approximately 2.00, this amount was found to be 3.292 gm. $KHSO_4$. By repeating the extraction with this concentration of

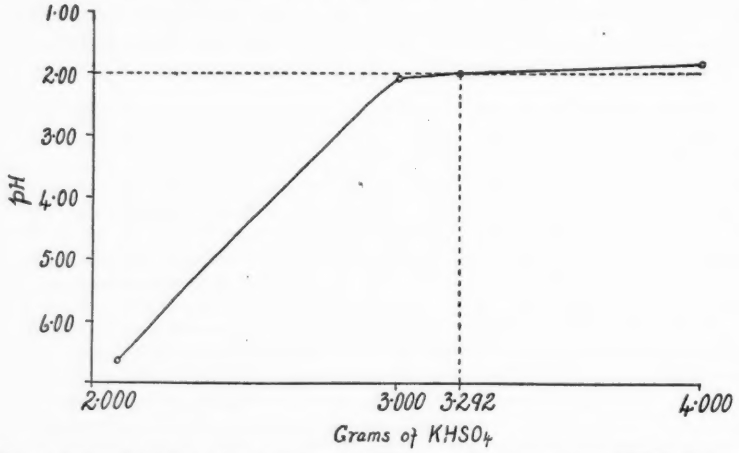

FIG. 4. INTERPOLATION OF THE AMOUNT OF $KHSO_4$ TO BE ADDED TO THE $KHSO_4$ SOLVENT IN ORDER TO FINISH AT A pH OF 2.00 IN EXTRACTING A CALCAREOUS SOIL SAMPLE

$KHSO_4$ in the solvent, a pH of 2.03 was obtained in the extract, and 282 mgm. P per 1,000 gm. of soil was found. The following table and figure 4 show how this operation is done.

$KHSO_4$ IN 400 CC. SOLVENT	pH OF EXTRACTS	P PER 1,000 GM. SOIL
gm.		*mgm.*
2.092	6.63	11
3.092	2.09	..
4.092	1.85	..
3.292	2.03	282

With a little experience, this operation takes very little time.

When working with the extraction of readily soluble phosphate from soils, acidity control is absolutely necessary for the interpretation of the results. From the determination of the

[12] If extracting a large number of calcareous soil samples, the use of two stock solutions of these concentrations of $KHSO_4$ could be employed.

pH values in the *extracts*, the quinhydrone electrode is rapid and, from the experience obtained so far in this laboratory, is also reliable.

The whole operation in determining readily soluble phosphate in a soil sample, extraction, filtering, and colorimetric determination can be done within half an hour, and a routine analyst can determine readily soluble phosphate in about 20 soil samples a day.

SUMMARY

Readily soluble soil phosphate is defined as the amount of phosphate extracted—expressed as milligrams P per 1,000 gm. of soil—by shaking 2 gm. soil (20 mesh) for 5 minutes, with a $KHSO_4$ solution of such a strength that the acidity in the filtered extracts is approximately 2.00.

An acid solvent of this strength has proved to be strong enough to extract the readily soluble phosphate in a soil sample; it does not break up the difficulty soluble soil phosphate appreciably; and the acidity of the extracts is high enough to prevent re-precipitation of dissolved phosphate.

The readily soluble phosphate in a soil sample is adequately removed by 5 minutes of shaking.

A method for extraction of readily soluble phosphate from soils by means of dilute $KHSO_4$ at a pH of 2.00 is described.

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STUDIES ON READILY SOLUBLE PHOSPHATE IN SOILS: II. THE VERTICAL DISTRIBUTION OF READILY SOLUBLE PHOSPHATE IN SOME REPRESENTATIVE ONTARIO SOILS[1]

H. W. LOHSE AND G. N. RUHNKE[2]

Ontario Agricultural College[3]

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It has been well established, that the roots of most cultivated plants, under natural conditions, penetrate into the lower horizons of the soil (20, 21). Undoubtedly, the plants do, in many cases, absorb nutrients from the lower soil horizons. Because of this, investigations on readily soluble nutrients from soils should not be restricted to the surface or cultivated layer alone, but should include all the subsequent horizons.

The purpose of this paper is to present some typical data on the vertical distribution of readily soluble phosphate in some typical soil horizons of both virgin and cultivated soils from Ontario, in the brown forest and podzol groups. The reason for publishing these preliminary results is partly for the purpose of giving some further data supporting the extraction method described elsewhere (9), and partly to call attention to the striking results obtained by extraction of readily soluble soil phosphates at a controlled pH value.

REVIEW OF LITERATURE

Only very few investigations reported in the literature are of the same nature and scope as the investigations presented here.

A large number of references deal with the distribution of the total amount of phosphorus in the different horizons of soils (1, 2, 6, 7, 10, 13, 14, 23, 24). Jenny (7) has given an excellent description of the distribution of the different elements (total amounts) in typical regional profiles in the United States. With respect to the total phosphorus content and its distribution in the profile, the analysis given for the podzol profile (7, p. 175) is most striking. The A_2 horizon of maximum leaching is almost devoid of this element. It must be admitted, however, that the total analysis gives very little information in regard to the nature and the movement of the soil phosphates in the horizons.

In studies of the water-soluble constituents in loess soils in Nebraska by Upson and Calvin (19), no regularity in the distribution of water-soluble phosphates was found. In studies of soils from the same region by Alway and Isham (1), it was shown that the amount of soil phosphates dissolved in 1 per cent citric acid extracts in the humid eastern area increased markedly from the first to the sixth foot, whereas in the most westerly arid portion a marked

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[2] Research assistant and assistant professor, department of chemistry, respectively.

[3] Published with the permission of the president.

decrease from the surface downward was found. It is important to note that the presence of carbonates in the subsoils from the western arid area interfered with the citric acid extraction. When carbonates were destroyed before extraction, the sixth foot horizon contained as much phosphate soluble in 1 per cent citric acid as did the first foot horizon.

Dunnewald (4) using 0.001 *N* H_2SO_4 has studied the distribution of "available" phosphorus in some Wyoming soil profiles. He concluded that "available" phosphorus decreases in the lower soil zones of all the profiles studied, as compared with the surface zones, where most of the organic matter has accumulated. This was true in basic soils as in those with acid reaction. He observed that in the basic soils the amount of lime increases in the lower zone to such an extent that the action of the 0.001 *N* acid is entirely neutralized, resulting in lowered values for "available" phosphorus. In a more recent paper (5) Dunnewald concludes that "available" phosphorus is more rapidly lost from the surface from grass soils than from podzolized timber soils.

Nemec (12) found that the humus layer in forest soils is the most abundant source of easily soluble phosphate, and in the lower layers there is a general parallelism between the citric acid solubility of soil phosphate and the humus content. The humus layer under pines is relatively low in citric acid-soluble phosphate. Much of the damage caused by removal of the débris of leaves for litter may be ascribed to the resulting decrease in the content of "available" phosphate in the soil.

Spurway (15) found, in Michigan soils, that fertilized muck soils retained nearly all of their water-soluble phosphate in the plowed portion, but in fertilized mineral soils, a downward penetration of water-soluble phosphate took place.

Stephenson and Chapman (16) working with California soils, have shown that readily soluble phosphate in the soil can penetrate appreciably below the surface foot in light to medium textured soils. Little or no penetration seems to take place in very heavy soils. The experiments indicated also that readily soluble phosphate from manure moves readily through the soil. Midgley (11) found that superphosphate, where applied as a surface dressing, moves downward very slowly. This downward movement is greatly influenced by different fertilizer salts.

In studies on Danish heath soils (podzols) Weis (22) found that they were very poor in phosphate extracted with nitric acid. In heavily podzolized sandy profiles, practically no phosphate could be extracted. An estimation of the distribution of the inorganic colloids showed that the largest amounts of inorganic colloids were accumulated in the B horizon.

In studies on southern Ontario soil profiles, Ruhnke (14) also has found that an accumulation of colloidal matter takes place in the B horizons.

The distribution of the fine material in soils might have great bearing upon the distribution and the nature of phosphates, as the phosphate content in the soil, after Lauterberg (8), is largely concentrated in the finer particles (less than 0.05 mm.).

EXPERIMENTAL METHODS

Sampling was done on the basis of natural horizons wherever possible. In the cultivated clayey soils, however, where it was difficult to distinguish the horizons, samples were taken at arbitrary depths corresponding to the changes in color and texture. The samples were air-dried on arrival at the laboratory, and when air-dried, passed through a 20-mesh sieve.

Reaction. The pH values of the soil samples were determined in 1:1 suspensions (CO_2-free distilled water used). In most cases the quinhydrone electrode was used, but in samples where the alkalinity was too high, colorimetric determinations were made.[4]

[4] All the samples have also been tested for their approximate pH value by a simple colorimetric test ranging from a pH of 4.4 to a pH of 7.6.

Readily soluble phosphate was extracted by means of dilute $KHSO_4$ at a pH of 2.0 (9); 2 gm. of air-dried soil sieved through a 20-mesh sieve was shaken 5 minutes with 400 cc. of a $KHSO_4$ solution at a pH of 2.0. Immediately after the elapse of 5 minutes the supernatant suspension was filtered rapidly through a 9½-cm. Buchner funnel. The phosphate content in the extracts was determined by the Deniges method, as worked out by Truog and Truog and Meyer (18), and somewhat modified by Chapman (3).

The term *readily soluble soil phosphate* refers to the amount of phosphate extracted from a soil sample by this procedure.

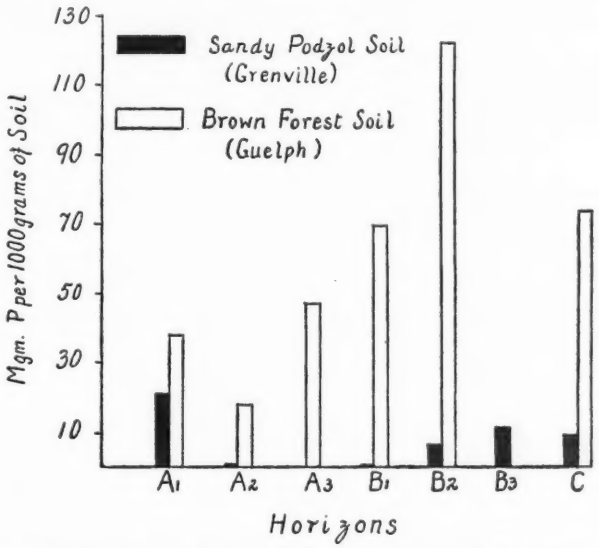

FIG. 1. THE DISTRIBUTION OF READILY SOLUBLE SOIL PHOSPHATE IN A TYPICAL SANDY PODZOL SOIL AND IN A TYPICAL BROWN FOREST SOIL

EXPERIMENTAL DATA

The experimental data obtained in the investigations dealt with in this paper are presented in tables 1, 2, 3, and 4. Figure 1 shows the distribution of readily soluble phosphate in a typical acid sandy podzol soil and in a typical alkaline brown forest soil profile.

DISCUSSION

Virgin podzol soils (table 1). The profiles are acid in all horizons. The relatively high pH value, 6.91, in the A_1 horizon from the Caledon profile is most likely due to material transferred up to the surface from a deeper layer, as this profile was sampled from a road cut.

The *sandy* podzols are distinguished by their very low content of readily soluble phosphate. It is interesting to note in this connection that Weis (22) found almost no phosphate by acid extraction of podzolized sandy heath profiles in Denmark. It will be seen that the surface layer contains the highest amount of phosphate. Dunnewald (4) found the same in acid profiles.

The *clayey* podzols are much richer in readily soluble phosphate in all horizons, although they are acid in reaction, and they have been vigorously leached.

TABLE 1

Reaction and readily soluble phosphate in virgin podzol soils

LOCATION	HORIZON	DEPTH	pH	P PER 1,000 GM. SOIL	REMARKS
		inches		*mgm.*	
1. South Gower Grenville	A_1	0–4	5.35	21	Sandy podzol developed on outwash plain material under predominantly coniferous forest with some hardwoods
	A_2	4–7	5.02	None	
	B_1	7–16	4.77	None	
	B_2	16–26	5.47	6	
	B_3	26–36	5.67	11	
	C	36–44	5.77	9	
2. Peel County Caledon Highland	A_1	0–3	6.91	17	Sandy loam podzol developed on morainic material covered with mixed forest
	A_2	3–4	4.83	7	
	A_3	4–7	5.91	None	
	B_1	7–13	6.10	6	
	B_2	13–16	6.10	11	
	C	16–30	5.97	14	
3. Warren Sudbury	A_1	.....	5.63	53	Clayey podzol on smooth till plain area. Sudbury district, northern Ontario
	A_2	.....	5.49	36	
	B_1	.....	5.55	44	
	B_2	.....	5.63	168	
	B_3	.....	6.12	225	
	C	.....	6.53	540	
4. Cochrane district Monteith	A_1	.....	5.81	125	Clayey podzol on gently rolling till plain area
	A_2	.....	4.70	19	
	B_1	.....	5.18	72	
	B_2	.....	5.50	204	
	C	.....	6.64	285	

The high content of readily soluble phosphate in the parent material in the clayey podzols is remarkable. Undoubtedly, the contrast between clayey and sandy podzols is partly explained by the great difference in the character of the parent materials from which these soils have developed.

Virgin brown forest soils (table 2). The content of readily soluble phosphate throughout the profiles is of the same magnitude level as found in the clayey podzols. All the soils presented are typical brown forest soils.

In the pronounced acid A horizons of the Kent profile (no. 3) the content of

readily soluble phosphate is very low in spite of the fact that the content in the parent material is high. Numbers 1 and 3 have the highest content of readily soluble phosphate in the parent material, numbers 2, 4, 5 in the B horizons. An accumulation of readily soluble phosphate in the B horizons may be due to the relatively high content of colloidal material found in this horizon (8, 14, 22). A high content of readily soluble phosphate in the parent material is explainable

TABLE 2

Reaction and readily soluble phosphate in virgin brown forest soils

LOCATION	HORIZON	DEPTH	pH	P PER 1,000 GM. SOIL	REMARKS
		inches		*mgm.*	
1. Beverly Wentworth Co.	A_1	0–5	6.98	27	Well-drained silt loam, developed on lacustrine deposit in glacial lake plain. Cover grass
	A_2	5–9	7.03	16	
	B	9–20	7.26	342	
	C	20–30	8.02	516	
2. Caledon East Peel Co.	A_1	0–3	7.69	27	Well-drained loam, developed in till plain area. Cover grass
	A_2	3–11	7.14	120	
	B	11–22	7.27	253	
	C	22–30	8.13	140	
3. MacGregor Creek Kent Co.	A_1	0–4	5.90	9	Moderately well-drained silt loam, developed on lacustrine deposit in glacial lake plain. Cover grass
	A_2	4–8	5.77	10	
	A_3	8–13	5.51	8	
	B_1	13–22	6.28	63	
	B_2	22–28	7.75	218	
	C_1	28–33	7.77	270	
	C_2	33–40	7.87	282	
4. Guelph Wellington Co.	A_1	0–5	7.94	38	Well-drained upland silt loam, on limestone drift. Cover grass
	A_2	5–11	7.68	18	
	A_3	11–19	7.68	47	
	B_1	19–28	7.66	69	
	B_2	28–36	8.27	122	
	C	36–42	8.01	73	
5. Beverly Wentworth Co.	A_1	0–5	7.33	20	Well-drained upland loam on limestone drift. Cover grass
	A_2	5–12	7.23	51	
	B	12–23	7.81	160	
	C	23–34	8.16	80	

from the fact that this horizon has a very high content of Ca. When such C horizons are being extracted with dilute acids, large amounts of dissolved Ca can be detected, whereas the extracts from A and B horizons contain only small amounts of dissolved Ca.

Cultivated brown forest soils (table 3). The striking feature in this group is the high content of readily soluble phosphate in the lower horizons. Numbers

TABLE 3

Reaction and readily soluble phosphate in cultivated brown forest soils

LOCATION	DEPTH	pH	P PER 1,000 GM. OF SOIL	REMARKS
	inches		*mgm.*	
1. Pain Court Kent Co.	0–10	7.38	183	Very productive dark brown soil. Corn, sugar beets, alfalfa, beans, wheat. Never fertilized
	10–18	8.00	204	
	18–28	8.12	213	
2. Ringold Kent Co.	0–6	7.28	142	Very productive black soil. Corn, sugar beets, alfalfa, grain, grown on drained areas. Never fertilized
	6–12	7.16	132	
	12–20	7.17	186	
	20–30	7.41	255	
3. Caledon E. Peel Co.	0–8	7.91	70	Gray-brown loam. Alfalfa area. Stand of alfalfa excellent—drainage good
	8–16	8.16	450	
	16–24	8.15	378	
4. Bolton Peel Co.	0–8	5.70	29	Gray-brown clay loam—typical alfalfa soil. Peel Co. Received 175 pounds 2-8-4 fertilizer, 1931. No previous fertilizer treatment
	8–16	7.35	277	
	16–25	8.13	550	
5. Brampton Peel Co.	0–8	6.08	14	Gray-brown clay loam-14-year-old alfalfa sod—plants vigorous but stand thinning out. Farmyard manure used 10 years ago, but no commercial fertilizer
	8–18	5.82	114	
	18–27	6.39	325	
6. Rainham Haldimand	0–6	5.18	13	Light gray-brown clay-rotation pasture. Canadian bluegrass. Farmyard manure in rotation but no commercial fertilizer ever used. Cultivated about 100 years
	6–14	6.46	30	
	14–22	8.07	475	
	22–30	7.97	420	
7. Charing Cross Kent Co.	0–8	5.89	17	Light gray-brown clay loam. Reverted to permanent pasture. Canada bluegrass. Little white clover. Never fertilized
	8–16	5.80	10	
	16–24	6.08	9	
	24–32	7.40	123	
	32–40	7.63	280	
8. Rainham Haldimand	0–8	4.85	None	Similar to Rainham soil above. Superphosphate gives very marked results where used on wheat on this farm
	8–16	4.89	10	
	16–24	6.87	200	
	24–32	7.78	410	

1, 2, and 3 are alkaline in all horizons and they have also a very high content of readily soluble phosphate. They are all very fertile soils. Numbers 1 and 2 are typical sugar beet soils from Kent County. Numbers 4 and 5 are typical alfalfa soils from Peel County. It will be seen that they are very much ex-

hausted of readily soluble phosphate in the surface. Since alfalfa can grow well in spite of that, it is apparently due to the fact that the plants can get their supply of phosphate from the lower horizons. From experience in this laboratory, a content of 14 mgm. and 29 mgm. P per 1,000 gm. of soil, respectively, as extracted by the $KHSO_4$-method, is so low that it would mean failure of the alfalfa crop if no other sources of phosphate were available.

For the start of a new sown crop on soils, as nos. 4 and 5 (especially one of short duration and quick growth), phosphate fertilization seems to be advisable.

Numbers 6, 7, and 8 represent cultivated soils, with an extreme deficiency of readily soluble phosphate. The cover on the pasture where sample 7 was taken consisted of a very poor stand of Canada bluegrass. The livestock on the

TABLE 4

Extraction of readily soluble soil phosphate in calcareous C horizons ($KHSO_4$ method)

PROFILE	FIRST EXTRACTION		FINAL EXTRACTION		
	pH in extract	P per 1,000 gm. soil	$KHSO_4$ added*	pH in extract	P per 1,000 gm. soil
		mgm.	*gm.*		*mgm.*
No. 4 Table 1	2.20	180	0.850	2.03	285
No. 2 Table 2	5.50	9	1.400	2.05	140
No. 3 Table 2 C_1	7.11	44	2.000	2.00	270
No. 3 Table 2 C_2	6.63	11	1.200	2.03	282
No. 4 Table 2	2.33	56	0.700	2.02	73
No. 1 Table 3	2.21	136	0.900	2.03	213
No. 3 Table 3	3.01	118	1.000	2.02	378
No. 4 Table 3	3.40	46	1.100	2.04	550
No. 6 Table 3	5.58	None	1.500	2.01	420
No. 8 Table 3	5.73	8	1.500	2.04	410

* The amount of solid $KHSO_4$ added in order to finish the extraction at a pH of approximately 2.0 is determined by interpolation on the basis of two extra extractions with higher acidity of the solvent.

farms where samples 6 and 8 were taken suffered severely from mineral deficiency. It is worthy of note that in these soils, where phosphate deficiency is so marked, a low level of readily soluble phosphate is found in both surface and subsurface horizons.

Summarized, it can be stated for these representative cultivated soils, that nos. 1, 2, and 3, represent soils not phosphate deficient; nos. 4 and 5 represent soils where deep rooted crops, once established, will have an adequate phosphate supply; and nos. 6, 7, and 8 represent soils of extreme phosphate deficiency.

As far as samples from cultivated soils are concerned, the few examples presented here are taken as typical. Numerous samples from cultivated soils have been extracted for readily soluble phosphate by the $KHSO_4$-method up to the present date. Experience thus obtained, in comparison with other methods and field experience, have shown that a marked phosphate deficiency

in Ontario soils is found where subsequent layers have contained below 30 mgm. P per 1,000 gm. of air-dried soil. What limits one should propose as optimal, or rather as rational, for the different crops, on different soil types, under different climatic conditions, and by different systems of field management, can only be decided by future collaborative experimental work in the laboratory and in the field.

Table 4 gives the results of extractions of calcareous C horizons.

CONCLUSIONS

These investigations show conclusively that surface sampling alone, for the purpose of determining soil fertility, is inadequate. Soils 4 and 5 from the alfalfa section in Peel County are striking examples in this respect.

The importance of extracting all the horizons at the same pH value in the extracts is apparent. For the case of calcareous C horizons, no idea as to the content of readily soluble phosphate would have been obtained if they had not been extracted at the same pH value as the A and B horizons.

Weaver (20) and Weaver and Bruner (21) have conclusively shown, in their extensive studies on the root development of cultivated plants, that deeply rooted plants can benefit by the plant food contained in the lower soil horizons. It thus becomes apparent that the high amounts of readily soluble phosphate, as found in the lower soil horizons in a number of soils described in this paper, are of great importance for the supply of phosphate to deeply rooted plants, such as alfalfa, sweet clover, and sugar beets. The results from the profiles from the alfalfa section in Peel County (nos. 4 and 5, table 3), represent striking examples in this respect.

There might be some doubt as to the ability of the plants to utilize the phosphate sources in the alkaline calcareous parent material,[5] and when phosphate from the lower horizons can be utilized by deeply rooted plants, it is most likely that this absorption of phosphates takes place from the B horizon.

It is a question of great importance in soil fertility studies, and it deserves further study.

SUMMARY

Readily soluble soil phosphate, extracted by 5 minutes of shaking with a $KHSO_4$ solution at a pH of 2.0, varies much in vertical distribution. Therefore, surface sampling alone is inadequate.

[5] Recent investigations of McGeorge and Breazeale (Tech. Bul. 35 and 36, Univ. of Ariz., Agr. Exp. Sta., 1931) have shown, that highly calcareous soils may have a high content of readily soluble phosphate, as determined by the methods of Dyer and Truog, in spite of the fact that these soils were phosphate deficient from an agricultural standpoint. The same authors have also shown that a high content of bicarbonate checks the phosphate absorption of plants. It thus becomes apparent that acid extractions of phosphate from soils high in carbonates (in arid regions) are of a very limited value. Soils from the humid regions, however, where the carbonates are leached out of the layers where the plants most likely absorb the phosphate acid extractions, as described in this paper, give a very useful index to the stock of readily soluble phosphate in a given soil sample.

In determining the vertical distribution of readily soluble soil phosphate, extraction at the same pH value in the extracts from all the horizons, is desirable. The extracts must also be at a pH value where re-precipitation of dissolved phosphate is prevented.

Sandy podzol soils seem to be very low in readily soluble phosphate in all horizons, the parent material included.

Clayey podzols and brown forest soils contained large amounts of readily soluble phosphate in the B horizon and in the parent material.

Cultivated soils of the brown forest group contained, in all cases, considerable amounts of readily soluble phosphate in the lower horizons.

Extractions of profile samples from typical cultivated brown forest soils, well known as to their fertility, indicate that the extraction method ($KHSO_4$ method) used gives a good index to the fertility of these soils, as far as their phosphate supply is concerned.

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FURTHER STUDIES ON NITROGEN ABSORPTION FROM CULTURE SOLUTIONS: II. BUCKWHEAT[1]

A. L. STAHL AND J. W. SHIVE

New Jersey Agricultural Experiment Station

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The investigations described in the following pages deal with a quantitative study of the absorption of nitrogen by the roots of buckwheat plants (*Fagopyrum esculentum* Moench.) from culture solutions in which they were grown. This paper is the second of two publications dealing with this problem. The experimental work of the present study with buckwheat was carried out in connection with the earlier work with oats (5), and in precisely the same manner and by the same methods, with only such modifications in treatment and handling as are necessitated by the differences in the forms and the relative duration of the life cycles of the two species.

The experimental procedure, including methods of growing the plants, the transfer of cultures for the absorption tests, and the methods of chemical analyses were described in detail in the earlier paper (5), therefore need not be repeated here. Any deviation from the experimental procedure dealt with in the paper just cited will be described in connection with the presentation of experimental results.

As has been stated in the earlier publication, the purpose of these investigations was primarily to determine the relative activity of the plants with respect to the absorption of nitrogen in the two forms, NH_4 and NO_3, by the roots of plants at various stages throughout the active life cycle, when both these forms of nitrogen are simultaneously present in the culture solution and available to the plants.

EXPERIMENTAL RESULTS

Preliminary experiments

In connection with some preliminary experiments with oats (5), similar experiments were carried out with buckwheat. As before, some of the data obtained from these preliminary experiments, which were carried out without continuous renewal of the culture solution during the test intervals, will be presented for the purpose of comparing the results with those obtained from the later experiments in which use was made of the method of continuous solution renewal at constant rates (4) throughout all the absorption intervals.

[1] Journal Series paper of the New Jersey Agricultural Experiment Station department of plant physiology.

The plants were grown in the modified Tottingham solution $T_1R_3C_5$ (1) to the age of 18 days in 2-quart fruit jars of colorless glass, with continuous solution renewal. The plants were then transferred, as previously described (5) from the 2-quart jars to pint jars containing 500 cc. of the test solution, where they remained for the time of the test interval without continuous flow of the solution through the culture jar. At the end of the test interval the plants were returned to the original 2-quart culture jars, after the roots had been rinsed free from adhering solution with distilled water, the rinse water being retained with the solution for analysis to determine the quantities of nitrogen removed by the plants. Here the plants were grown as before until the next experimental period. This procedure was continued through the growth period of 58 days.

Since the plants were not harvested at the end of each test interval, dry weight yields were not obtained upon which to calculate rate absorption on the basis of nitrogen absorbed from solution per unit of dry plant material per unit of time. In these preliminary experiments, therefore, all results were calculated only on the quantity basis of nitrogen in the form in question absorbed from a fixed quantity of solution, in milligrams per culture per hour. These data are presented in table 1. Here again, as in the experiments with oats, 12 test intervals were employed during each experimental period. These ranged in time from 8 hours as the minimum to 96 hours as the maximum time during which the roots of the plants were exposed to the test solution. Each horizontal section of table 1 presents the data obtained during an experimental period of 96 hours. In making the absorption tests, duplicate cultures were employed and all analyses were made in duplicate; hence each value in the table except those in the last line of each horizontal section, represents the average of four determinations. Since duplicate cultures comprising three plants each were employed for each of the 12 test intervals, each of the values in the last line of the horizontal sections, marked *average*, represents the final average of 48 determinations involving 24 cultures and 72 plants.

The final average values for all the experimental periods as given in table 1 were plotted to form the graphs of figure 1. As indicated by the graph representing the course of quantity absorption of nitrogen as NH_4 per culture per hour during the growth cycle investigated, the maximum intake of nitrogen in this form occurred at the 18-day period, with rapid decline to a very low value at 30 days and thereafter to the stage of maturity and the formation of ripe seeds.

The graph representing the course of quantity absorption of nitrogen as NO_3 begins with relatively low values, rises to a maximum at 50 days, and then rapidly declines with approaching maturity. Thus, maximum quantity absorption of nitrogen as NO_3 occurs at a relatively late period in the growth cycle, whereas the maximum quantity absorption of nitrogen as NH_4 occurs early in the cycle, at the beginning of the blossoming period.

It will be observed that maximum quantity absorption of nitrogen as NO_3 is

TABLE 1

Quantity absorption of nitrogen as NH_4, NO_3, and total nitrogen per culture by buckwheat plants during absorption intervals ranging in time from 8 hours to 96 hours, at different stages of development in the life cycle

AGE OF PLANTS	ABSORPTION INTERVALS	QUANTITY OF NITROGEN ABSORBED PER CULTURE					
		As NH_4		As NO_3		Total nitrogen	
		During absorption interval	Per hour	During absorption interval	Per hour	During absorption interval	Per hour
days	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
	8	3.764	0.471	−0.178	−0.022	3.586	0.449
	16	5.839	0.365	−0.799	−0.050	5.040	0.315
	24	5.426	0.226	−0.711	−0.030	4.715	0.196
	32	5.952	0.186	3.594	0.112	9.546	0.298
	40	7.439	0.186	3.594	0.090	11.033	0.276
	48	7.527	0.157	5.863	0.122	13.390	0.279
18	56	7.089	0.127	6.573	0.117	13.662	0.244
	64	7.264	0.114	7.106	0.111	14.370	0.225
	72	5.962	0.083	7.595	0.105	13.557	0.188
	80	5.426	0.068	10.393	0.130	15.819	0.198
	88	6.127	0.070	10.393	0.118	16.520	0.188
	96	6.714	0.070	9.060	0.094	15.774	0.164
Average			0.177		0.075		0.252
	8			1.534	0.192	1.534	0.192
	16	−0.120	−0.008	2.941	0.184	2.821	0.176
	24	−0.297	−0.012	4.459	0.186	4.162	0.174
	32	0.221	0.007	3.718	0.116	3.939	0.123
	40	0.332	0.008	5.903	0.148	6.235	0.156
	48	0.703	0.015	7.581	0.158	8.284	0.173
30	56	1.295	0.023	7.834	0.140	9.129	0.163
	64	1.444	0.023	8.198	0.128	9.642	0.151
	72	2.853	0.040	8.272	0.115	11.125	0.155
	80	3.333	0.042	7.977	0.100	11.310	0.142
	88	4.296	0.049	8.162	0.093	12.458	0.142
	96	4.296	0.045	8.656	0.090	12.952	0.135
Average			0.019		0.138		0.157
	8	−0.410	−0.051	4.525	0.566	4.115	0.515
	16	−0.602	−0.038	5.434	0.340	4.832	0.302
	24	−0.602	−0.025	5.842	0.243	5.240	0.218
	32	0.121	0.004	6.251	0.195	6.375	0.199
	40	0.396	0.010	6.673	0.167	7.069	0.177
	48	1.106	0.023	7.785	0.162	8.891	0.185
42	56	1.699	0.030	8.360	0.149	10.059	0.179
	64	1.890	0.030	9.012	0.141	10.902	0.171
	72	2.274	0.032	9.702	0.135	11.976	0.167
	80	2.312	0.029	10.392	0.130	12.704	0.159
	88	2.235	0.025	10.734	0.122	12.969	0.147
	96	1.697	0.017	11.006	0.115	12.685	0.132
Average			0.027		0.205		0.213

TABLE 1—*Concluded*

AGE OF PLANTS	ABSORPTION INTERVALS	QUANTITY OF NITROGEN ABSORBED PER CULTURE					
		As NH_4		As NO_3		Total nitrogen	
		During absorption interval	Per hour	During absorption interval	Per hour	During absorption interval	Per hour
days	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
50	8	−0.395	−0.049	4.162	0.520	3.767	0.471
	16	−0.488	−0.031	5.778	0.361	5.290	0.330
	24	−0.244	−0.010	6.952	0.290	6.708	0.280
	32	0.000	0.000	6.757	0.211	6.757	0.211
	40	0.242	0.006	7.883	0.197	8.125	0.203
	48	1.076	0.022	8.519	0.177	8.595	0.199
	56	0.981	0.018	8.568	0.153	9.549	0.171
	64	1.322	0.021	9.106	0.142	10.428	0.163
	72	1.764	0.025	9.106	0.126	10.870	0.151
	80	1.373	0.017	9.694	0.121	11.067	0.138
	88	1.960	0.022	10.183	0.116	12.143	0.138
	96	2.058	0.021	10.183	0.106	12.241	0.127
Average			0.005		0.210		0.215
58	8	−0.243	−0.030	1.064	0.133	0.821	0.103
	16	−0.488	−0.031	1.162	0.073	0.674	0.042
	24	0.099	0.004	1.455	0.061	1.554	0.065
	32	0.639	0.020	2.139	0.067	2.778	0.087
	40	1.472	0.037	2.579	0.064	4.051	0.101
	48	1.374	0.029	3.068	0.064	4.442	0.093
	56	1.570	0.028	3.605	0.064	5.175	0.092
	64	1.565	0.024	3.556	0.056	5.121	0.080
	72	1.766	0.025	4.192	0.058	5.958	0.083
	80	1.472	0.018	5.249	0.066	6.721	0.084
	88	1.962	0.022	5.364	0.061	7.326	0.083
	96	1.962	0.020	6.049	0.063	8.011	0.083
Average			0.014		0.069		0.083

somewhat higher than maximum quantity absorption as NH_4. However, since the former occurs at a time when the plants are relatively large and the latter when the plants are small, the NO_3-rate of absorption at the point of maximum quantity absorption is very much lower than the NH_4-rate at the point of maximum quantity absorption. This will be clearly brought out in connection with the presentation of the data on rate absorption calculated on the basis of milligrams of nitrogen absorbed per gram of dry plant material per hour.

Quantity absorption of total nitrogen as indicated by the course of the upper graph of figure 1, is determined while the plants are young by quantity absorption of nitrogen as NH_4, and during the late growth phases by quantity absorption of nitrogen as NO_3. This is in direct agreement with the results obtained

with oats. It also harmonizes with the work of Naftel (2), who found that cotton seedlings before they were from 3 to 5 weeks old absorbed more NH_4 nitrogen than NO_3 nitrogen; but after this age more NO_3 nitrogen was absorbed in a given time than NH_4 nitrogen. This graph also indicates a secondary maximum point for quantity absorption of total nitrogen, which occurs late in the growth cycle (50 days) and is determined by quantity absorption of nitrogen as NO_3.

As in the studies with oats, so again in these preliminary experiments with buckwheat, it is important to point out the fact, as indicated by the data of

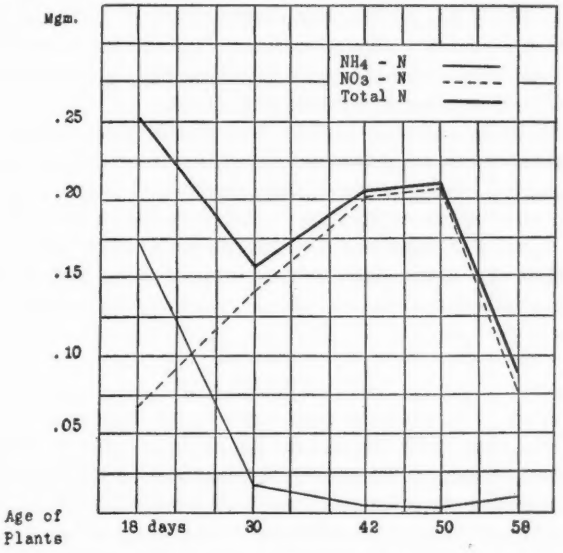

FIG. 1. GRAPHS REPRESENTING QUANTITY ABSORPTION OF NITROGEN AS NH_4, NO_3, AND TOTAL NITROGEN IN MILLIGRAMS PER CULTURE PER HOUR AT DIFFERENT PERIODS IN THE ACTIVE LIFE CYCLE OF THE BUCKWHEAT PLANT

table 1, that without continuous renewal of the culture solution during the test intervals, quantity absorption per culture per hour decreases very markedly with increase in the time duration of the absorption interval, when the roots are exposed to a fixed, relatively small quantity of solution (500 cc.). This influence of the duration of the test intervals upon absorption is negligible only when the absorption rates are very low and when the roots are exposed to comparatively large volumes of test solution. A thorough discussion of this phenomenon was presented in the earlier publication dealing with oats, and since the results obtained with buckwheat, if somewhat more pronounced, were similar to those obtained with oats, it is scarcely necessary here to emphasize further the importance of some adequate continuous flow system of solution

renewal during investigations of the nature here described, and for the growth of experimental plants in general where plant nutrition studies with culture solutions are involved.

The graphs of figure 1 represent the course of quantity absorption of nitrogen in the two forms only from the beginning of the blossoming period (18 days) to maturity; and without continuous flow of solution during the test intervals. Since the buckwheat plant enters the blossoming phase early in the life cycle and even before its vegetative development is in the advanced stages, it was interesting and important here to obtain a record of the course of nitrogen absorption during this early development from germination to flowering. To accomplish this it was necessary to develop a special technique involving a large number of plants to obtain absorption data with any degree of accuracy because of the small size and low quantity of nitrogen absorbed by individual plants. This is described in the following section.

Again in the data of table 1, as in the preliminary experiments with oats, some negative values are indicated for quantity absorption of nitrogen, both as NH_4 and NO_3 during several of the experimental periods. As previously stated (5) no adequate explanation of this phenomenon can here be offered. Suffice it to say that negative absorption (excretion) never occurred when the test solutions were continuously renewed during contact with the plant roots, as was done in the investigation of the *absorption series* described in the following sections.

The Absorption Series

Introductory. The plants of these series were harvested at the end of the experimental period with the termination of the absorption tests, and their dry weights obtained. This required a separate series of plants for the tests made during each experimental period. Seven such series of plants were grown and tested during the active life cycle. Each series consisted of six cultures which were conducted in duplicate. Tests were made at 4-day intervals up to the time the plants began to bloom at 18 days.

Because of their small size during the very early growth periods and the small quantities of nitrogen absorbed in a given time by individual seedlings, it was necessary to employ a large number of plants in a culture to provide for the removal by absorption of sufficient quantities of nitrogen from the culture solution to insure a fairly accurate measure by chemical analysis of the nitrogen so removed. To accomplish this, seeds were germinated between wet blotters in a moist chamber. A large number of strongly germinating seeds were then selected and placed on a germinating net tightly stretched over a small granite-ware pan in the manner described by Shive (3), the pan being filled with the culture solution. Here the plants were grown with continuous solution renewal up to the time when the absorption tests were begun. In preparation for the absorption test the old solution was drained from the pan, the plant

roots and pan were rinsed with distilled water and a measured quantity, 500 cc., of test solution was poured into the pan, the roots of the seedlings which protruded through the net being immersed. At the end of the test interval the net was removed from the pan, and the roots were rinsed with distilled water, which was retained with the solution for analysis. The total dry weight value of the plants from each pan was divided by the number of seedlings in the pan and multiplied by 3 to obtain the average weight of three plants, which in all cases is taken to represent the number of plants per culture upon which all calculations are based. Plants to be tested after the age of 14 days were mounted in paraffined cork stoppers as described by Tottingham (6), three plants to each culture, and their absorption was determined in the manner previously described (5).

The modified Tottingham solution $T_1R_3C_3$ (1) which contained the four salts KH_2PO_4, $Ca(NO_3)_2$, $MgSO_4$, and $(NH_4)_2SO_4$ in the following volume molecular proportions 0.0021, 0.0043, 0.0071, and 0.0042, respectively, and with approximately equal proportions of nitrogen in the two forms, NO_3, and NH_4, was used as the growth medium for the plants and also as the test solution during each of the seven experimental periods.

Throughout the test intervals the roots of the plants of each culture were immersed in a fixed quantity, 500 cc., of solution which was continuously renewed by being passed through the culture vessel at a constant rate of flow 1,000 cc. of new solution during the respective test interval. The total quantity, 1,500 cc., of solution to which the roots of a culture were exposed during the test interval was used for the chemical analyses to determine the quantities of nitrogen removed from the solution by the plants.

The results of the chemical analyses were calculated on the quantity basis of nitrogen absorbed in the two forms NH_4 and NO_3 per culture, of three plants, per hour, as well as on the rate basis of nitrogen absorbed in the two forms per gram of dry plant material per hour. The results obtained by the two methods of calculation are presented and discussed separately.

Absorption of nitrogen per culture per hour. In table 2 are presented the data dealing with quantities of nitrogen in the two forms in milligrams per culture per hour absorbed from culture solutions by buckwheat plants of seven different series. This table is constructed in precisely the same manner as table 1, but as previously stated, data are shown in each horizontal section for six instead of twelve test intervals. Thus each of the values marked *average* in the table represents the final average value of 24 determinations involving 12 cultures and 36 plants. These final average values only will be considered in the discussion, and they are plotted to form the graphs of figure 2.

It will be observed that the graph representing the march of quantity absorption of nitrogen as NH_4 in milligrams per culture per hour, begins with a low value at the 2-day period and rapidly rises to a maximum at 18 days, which

TABLE 2

Quantity absorption of nitrogen as NH_4, NO_3, and total nitrogen per culture per hour by buckwheat plants during absorption intervals ranging in time from 8 to 48 hours, at different stages of development throughout the active life cycle

AGE OF PLANTS	TEST INTERVALS	QUANTITY OF NITROGEN PER CULTURE ABSORBED					
		As NH_4		As NO_3		Total nitrogen	
		During test interval	Per hour	During test interval	Per hour	During test interval	Per hour
days	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
2	8	1.168	0.146	0.000	0.000	1.168	0.146
	16	1.763	0.110	0.000	0.000	1.763	0.110
	28	2.820	0.118	0.000	0.000	2.820	0.118
	32	3.327	0.104	0.006	0.000	3.333	0.104
	40	4.164	0.104	0.011	0.000	4.175	0.104
	48	5.045	0.105	0.011	0.000	5.056	0.105
Average			0.115		0.000		0.115
6	8	1.765	0.221	0.281	0.035	2.046	0.256
	16	3.198	0.200	0.446	0.028	3.644	0.228
	24	5.084	0.212	0.681	0.029	5.775	0.241
	32	7.006	0.219	0.815	0.025	7.821	0.244
	40	8.195	0.205	0.991	0.025	9.186	0.230
	48	8.944	0.186	1.261	0.026	10.205	0.212
Average			0.207		0.028		0.235
10	8	3.265	0.408	0.475	0.059	3.740	0.467
	16	6.368	0.399	0.594	0.037	6.976	0.436
	24	8.635	0 360	0.892	0.037	9.527	0.397
	32	11.309	0.353	1.247	0.039	12.556	0.392
	40	14.129	0.353	1.930	0.048	16.059	0.401
	48	17.395	0.362	2.078	0.043	19.473	0.402
Average			0.373		0.044		0.417
14	8	5.699	0.712	0.593	0.074	6.292	0.786
	16	8.786	0.549	1.247	0.078	10.033	0.627
	24	11.161	0.465	1.544	0.064	12.705	0.529
	32	13.595	0.425	1.900	0.059	15.495	0.484
	40	15.317	0.383	1.900	0.048	17.217	0.431
	48	19.098	0.398	1.900	0.040	20.998	0.438
Average			0.489		0.060		0.549
18	8	5.646	0.706	0.540	0.068	6.186	0.774
	16	8.139	0.509	2.130	0.133	10.269	0.642
	24	11.159	0.465	5.390	0.225	16.549	0.690
	32	17.885	0.559	5.390	0.168	23.275	0.727
	40	18.380	0.460	8.793	0.220	27.173	0.680
	48	20.144	0.420	9.860	0.205	30.004	0.625
Average			0.520		0.170		0.690

TABLE 2—*Concluded*

AGE OF PLANTS	TEST INTERVALS	QUANTITY OF NITROGEN PER CULTURE ABSORBED					
		As NH_4		As NO_3		Total nitrogen	
		During test interval	Per hour	During test interval	Per hour	During test interval	Per hour
days	*hours*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
38	8	0.360	0.045	5.433	0.679	5.793	0.724
	16	1.190	0.074	9.377	0.586	10.567	0.660
	24	1.659	0.069	11.676	0.487	13.335	0.556
	32	2.547	0.080	13.518	0.422	16.065	0.502
	40	2.835	0.071	15.588	0.390	18.423	0.461
	48	3.411	0.071	16.743	0.349	20.154	0.420
Average			0.068		0.486		0.514
58	8	0.479	0.060	2.340	0.293	2.819	0.353
	16	1.031	0.064	5.408	0.338	6.439	0.402
	24	1.104	0.046	5.334	0.222	6.438	0.268
	32	1.178	0.037	6.288	0.197	7.466	0.234
	40	1.325	0.033	8.046	0.201	9.371	0.234
	48	1.472	0.031	9.072	0.189	10.544	0.220
Average			0.045		0.240		0.285

marks the beginning of the blossoming period. Thereafter the graph takes a steep downward slope and shows very low values for quantity absorption of nitrogen as NH_4 after the plants had passed through the blossoming stage.

The graph showing the march of quantity absorption of nitrogen as NO_3 in milligrams per cultuer per hour, starts from zero at the 2-day period, shows very low values during the early development of the plants up to the beginning of the blossoming period, after which the graph rises rapidly to a maximum point corresponding to a period considerably after the plants had passed the reproductive phase of development. Thereafter the graph takes a gradual downward slope, indicating continuously declining values as the plants approach maturity.

The course of the graph representing quantity absorption of total nitrogen shows a very steep upward slope to a maximum point which is determined by the absorption of nitrogen as NH_4 and corresponds to the maximum point in the graph representing the absorption of nitrogen as NH_4. Following the maximum point the graph takes a continuously downward slope, its course in the late phases of the life cycle being determined by the absorption of nitrogen as NO_3.

A comparison of the graphs of figure 2 with the corresponding graphs representing quantity absorption of nitrogen by the oat plant under like experimental conditions, brings out some interesting similarities as well as some significant differences between the behavior of the two species with respect to

the quantity absorption of nitrogen in the two forms here considered. The main point of similarity in the two species lies in the fact that both species utilize NH_4-nitrogen as the main source of supply of this element during the early stages of development, and NO_3-nitrogen as the main source of supply during late stages of growth. Both species show the period of maximum quantity absorption of total nitrogen during the blossoming period, but in the buckwheat series this point corresponds to, and is determined by, the point of

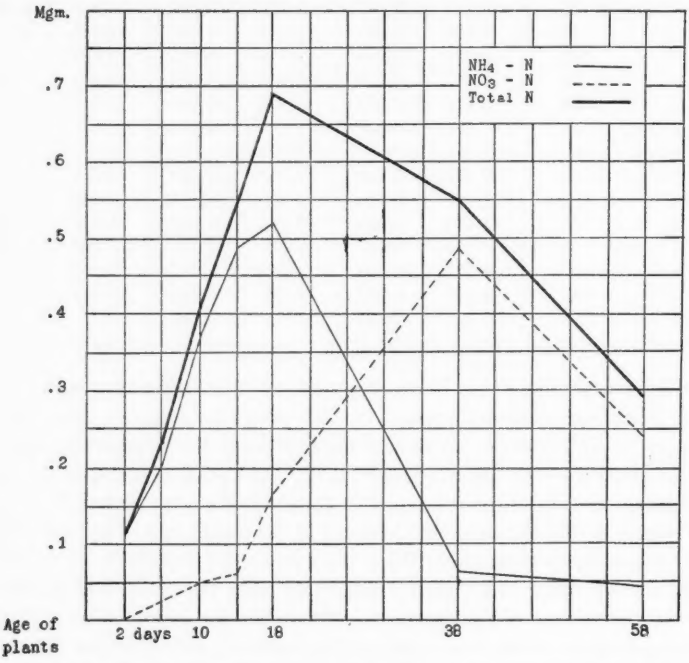

FIG. 2. GRAPHS REPRESENTING QUANTITY ABSORPTION OF NITROGEN AS NH_4, NO_3, AND TOTAL NITROGEN IN MILLIGRAMS PER CULTURE PER HOUR AT DIFFERENT PERIODS THROUGHOUT THE ACTIVE LIFE CYCLE OF THE BUCKWHEAT PLANT

maximum quantity absorption of NH_4-nitrogen; whereas in the oats series it corresponds to, and is determined by, the point of maximum absorption of NO_3-nitrogen.

In the buckwheat series, maximum quantity absorption NH_4-nitrogen occurs during the blossoming period, in the oats series it occurs at a much earlier stage. In the buckwheat series, maximum quantity absorption of NO_3-nitrogen occurs some time after the plants have passed through the blossoming period, in the oats series it occurs during the blossoming period. Thus from a comparison of the graphs representing the march of quantity absorption

of nitrogen in these two species it is apparent that quantity absorption of NH_4-nitrogen plays a much more pronounced rôle in the buckwheat series than it does in the oats series, and for more than half the active life cycle it is the predominating factor in determining quantity absorption of total nitrogen. On the other hand, quantity absorption of NO_3-nitrogen plays a much more pronounced rôle in the oats series than it does in the buckwheat series, and during nearly two-thirds of the active life cycle of the oat plant it is the predominating factor in determining quantity absorption of total nitrogen in this species, when the two forms of nitrogen are simultaneously present in the culture solutions in approximately equal proportions.

Absorption of nitrogen per gram of dry plant material per hour (rate absorption). The data relating to absorption rates calculated on the basis of nitrogen in the different forms absorbed per gram of dry plant material per hour, are presented in table 3. As before, only the final average values are considered in the discussion of these data. The final average values representing nitrogen absorbed as NH_4 and NO_3 in milligrams per gram of dry plant material per hour are designated the *NH_4-rate* and the *NO_3-rate*, respectively, for the sake of convenience. The final average rate values as given in table 3 were plotted to form the graphs of figure 3.

The graph representing the course of the NH_4-rate during the active life cycle begins by indicating high values even at the 2-day period, but the values increase to a maximum point at the 10-day period. Thereafter the graph takes a steep downward slope to a very low average value at the 38-day period. After the 38-day period the NH_4-rates are practically negligible.

The graph showing the course of the NO_3-rate begins by indicating a zero value at the 2-day period. The graph rises to a maximum at the 18-day period, which marks the beginning of the blossoming stage, and indicates values which are only slightly below the maximum throughout the entire reproductive period. After this, there is a very gradual decline with approaching maturity. However, it will be observed that the maximum NO_3-rate is relatively very low compared with the maximum NH_4-rate and occurs at a later period in the life cycle.

The graph representing the average rates of total nitrogen absorption is determined through the first half of the growth cycle by the NH_4-rates, and therefore takes the form of the NH_4-rate graph during these periods. It is only during the later phases of growth that the NO_4-rates exceed the NH_4-rates and only during that portion of the cycle when the rates of total nitrogen absorption are relatively very low.

A comparison of the rate graphs of figure 3 with the corresponding ones previously published (5) representing the course of the rates of nitrogen absorption by the oat plant, shows a marked similarity between the NH_4-rate graphs for the two species. Both indicate very high maximum values occurring during the early growth phases and very low values on approaching maturity. Likewise, the NO_3-rate graphs of the two species show remarkable

TABLE 3

Rate absorption of nitrogen as NH_4, NO_3, and total nitrogen per gram of dry plant material per hour by buckwheat plants during absorption intervals ranging in time from 8 hours to 48 hours, at different stages of development throughout the active life cycle

AGE OF PLANTS	TEST INTERVALS	DRY WEIGHT PER CULTURE (3 PLANTS)	ACTUAL NITROGEN ABSORBED PER GRAM DRY TISSUE PER HOUR		
			NH_4-N	NO_3-N	Total N
days	*hours*	*gm.*	*mgm.*	*mgm.*	*mgm.*
2	8	0.043	3.395	0.000	3.395
	16	0.045	2.444	0.000	2.444
	24	0.041	2.878	0.000	2.878
	32	0.046	2.261	0.000	2.261
	40	0.045	2.311	0.000	2.311
	48	0.046	2.283	0.000	2.283
Average		0.044	2.595	0.000	2.595
6	8	0.061	3.623	0.574	4.197
	16	0.059	3.390	0.475	3.865
	24	0.060	3.533	0.483	4.016
	32	0.065	3.369	0.385	3.574
	40	0.070	2.929	0.357	3.286
	48	0.075	2.480	0.347	2.827
Average		0.065	3.221	0.437	3.658
10	8	0.112	3.643	0.527	4.170
	16	0.105	3.800	0.352	4.152
	24	0.111	3.243	0.333	3.576
	32	0.114	3.096	0.342	3.438
	40	0.112	3.152	0.429	3.581
	48	0.113	3.204	0.381	3.585
Average		0.111	3.356	0.394	3.750
14	8	0.164	4.341	0.451	4.729
	16	0.150	3.660	0.520	4.180
	24	0.165	2.818	0.388	3.206
	32	0.180	2.361	0.328	2.689
	40	0.199	1.925	0.241	2.166
	48	0.191	2.084	0.209	2.293
Average		0.175	2.865	0.356	3.221
18	8	0.292	2.418	0.233	2.651
	16	0.300	1.697	0.443	2.140
	24	0.274	1.697	0.821	2.518
	32	0.308	1.815	0.545	2.360
	40	0.294	1.565	0.748	2.313
	48	0.310	1.355	0.661	2.016
Average		0.296	1.758	0.575	2.333

TABLE 3—*Concluded*

AGE OF PLANTS	TEST INTERVALS	DRY WEIGHT PER CULTURE (3 PLANTS)	ACTUAL NITROGEN ABSORBED PER GRAM DRY TISSUE PER HOUR		
			NH_4-N	NO_3-N	Total N
days	*hours*	*gm.*	*mgm.*	*mgm.*	*mgm.*
38	8	1.050	0.043	0.647	0.690
	16	1.123	0.066	0.523	0.589
	24	1.086	0.064	0.448	0.512
	32	1.152	0.069	0.366	0.435
	40	0.992	0.072	0.393	0.465
	48	1.078	0.066	0.324	0.390
Average		1.080	0.063	0.451	0.514
58	8	3.648	0.016	0.080	0.096
	16	3.726	0.017	0.091	0.108
	24	3.410	0.013	0.065	0.078
	32	3.406	0.011	0.058	0.069
	40	3.563	0.009	0.056	0.065
	48	3.485	0.009	0.054	0.063
Average		3.540	0.013	0.067	0.080

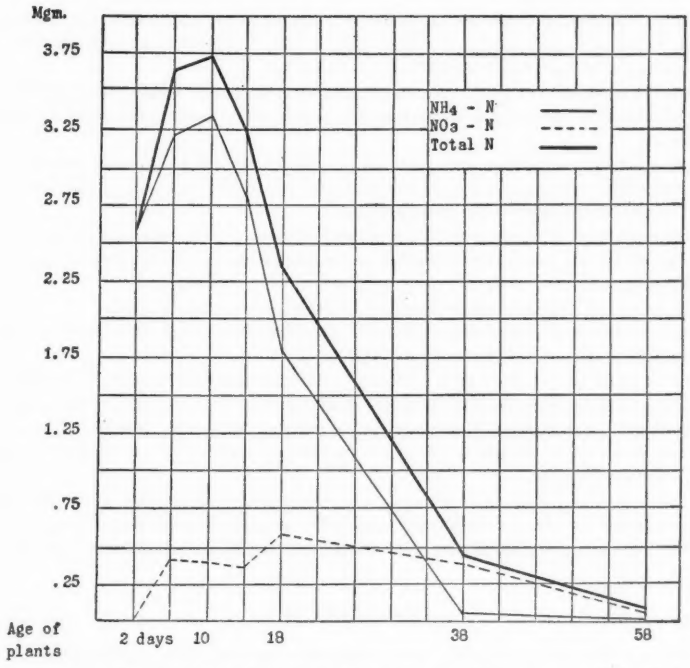

FIG. 3. GRAPHS REPRESENTING RATES OF ABSORPTION OF NITROGEN AS NH_4, NO_3, AND TOTAL NITROGEN IN MILLIGRAMS PER GRAM OF DRY PLANT MATERIAL, AT DIFFERENT PERIODS THROUGHOUT THE ACTIVE LIFE CYCLE OF THE BUCKWHEAT PLANT

agreement in that both begin with low values and reach their maximum points during the blossoming period. The comparison further shows that in the buckwheat series the NH_4-rate plays a greater rôle in the rate of total nitrogen absorption than it does in the oats series, and during more than half of the active life cycle of the buckwheat plant it is the predominating factor determining the rates of total nitrogen absorption. On the other hand, the NO_3-rate plays a more prominent rôle in the oats series than it does in the buckwheat series, and during more than half the active life cycle of the oat plant it is the predominating factor in determining the rate of total nitrogen absorption.

SUMMARY

Quantitative studies were made of the absorption of nitrogen by the buckwheat plant from culture solutions containing approximately equal proportions of nitrogen as NH_4 and NO_3, at various stages in the active life cycle of the plants. The main results of the investigations may be summarized briefly as follows:

Quantity absorption (milligrams per culture per hour) of nitrogen as NH_4 is at its maximum at the beginning of the blossoming period, declines rapidly after blossoming, and is low during the late stages of growth.

Quantity absorption of nitrogen as NO_3 is very low up to the beginning of the blossoming period, increases rapidly during the blossoming period, and reaches a maximum after the blossoming period, then declines rapidly.

Quantity absorption of total nitrogen attains a maximum point which corresponds to the point of maximum absorption of nitrogen as NH_4. This occurs at the beginning of the flowering period.

Rate absorption (milligrams per gram of dry plant tissue per hour) of nitrogen as NH_4 begins with high values and reaches a maximum early in the life cycle, and shows low values only during the late stages in the growth cycle.

Rate absorption of nitrogen as NO_3 is without value at a very early stage in the cycle, reaches a maximum during the blossoming period, then declines very slowly with approaching maturity.

The maximum NH_4-rate is approximately six times the maximum NO_3-rate.

Rate absorption of total nitrogen attains a maximum point which corresponds to, and is determined by, the point of the maximum NH_4-rate.

The NH_4-rate plays a much more prominent rôle in nitrogen absorption by the buckwheat plant than does the NO_3-rate, and during the greater part of the active life cycle it is the predominating factor in determining the rate of total nitrogen absorption. The NO_3 rates exceed the NH_4 rates only in the late stages of the growth cycle, when the rates of total nitrogen absorption are very low.

A comparison of buckwheat with oats, relative to nitrogen absorption, shows that NH_4-nitrogen absorption plays a much more prominent rôle in buckwheat than it does in oats, and NO_3-nitrogen absorption plays a much more pronounced rôle in oats than it does in buckwheat.

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THE INFLUENCE OF LEGUME VERSUS NON-LEGUME CROPS ON THE MICROBIOLOGICAL ACTIVITIES IN THE SOIL: II. NITRIFICATION AND CELLULOSE DECOMPOSITION[1]

JAMES E. FULLER

Massachusetts Agricultural Experiment Station

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A previous publication (2) from this experiment station has considered the influence of legume versus non-legume crops on the numbers of microörganisms in the soil of an experimental field, and on the distribution of nitrogen-fixing activity over the field. The present report considers nitrification and cellulose decomposition in the soil of the same field.

Waksman (7), after a series of studies of nitrification in field soils, stated that his results indicate that studies of nitrification can yield information for the differentiation of soil fertility as well as can the determination of numbers of microörganisms. Waksman and Starkey (8) stated that the carbon dioxide evolved from soils, both without and with addition of small amounts of organic matter, can be used in grading the soils on a basis of their fertility, as well as can the determination of numbers of microörganisms and nitrification in soils.

EXPERIMENTAL

The field from which the soil used in the present experiments was taken has been described previously (2). A review of that description is given here.

The field is divided from north to south into four strips, each of which is 2 rods wide and 12 rods long. During the seasons when legumes are planted the strip on the west side of the field is planted with a legume crop, the strip next to the east is planted with a non-legume crop, then follow in order a strip planted with the same legume crop as the first and a fourth strip with the same non-legume crop as the second. These strips are designated by the crops planted thereon as: Legume West, Non-legume West, Legume East, and Non-legume East.

The field is further divided from west to east into strips which are 2 rods wide and 8 rods long each. These transverse strips are numbered from 5 to 10 inclusive. Strips 1 to 4 inclusive are not in use.

The division of the field into longitudinal and transverse strips results in 24 plots, each 2 rods square.

Each season the field is treated uniformly over its whole area with sulfate of potash and with superphosphate. The liming of the soil is described in the aforementioned previous publication (2), and in an outline from Morse given in the following. The nitrogen treatment differs with respect to the numbered strips. In past seasons strip 5 has been treated with sodium nitrate; strip 6, with ammonium sulfate until 1922 and none since; strip 8, with ammonium sulfate; and strip 10, with dry ground fish. Strips 7 and 9 are check strips with no nitrogen treatment.

[1] Published as Contribution No. 154, Massachusetts Agricultural Experiment Station.

Professor F. W. Morse of the Department of Plant and Animal Chemistry of this Experiment Station, who is in charge of the experimental field, has furnished information concerning the treatment of the field and the crops planted since the period covered by the experiments already published (2). Professor Morse's statement follows:

1929. Nitrogen omitted from strips 5, 8, and 10. All plots fertilized with sulfate of potash and superphosphate as usual.
Grass and clover continued as crops from seeding of previous year.
1930. Fertilized as usual with sulfate of potash and superphosphate on all plots. Nitrogen applied to plots 5, 8, and 10.
Japanese millet seeded on all plots.
1931. 2,000 pounds ground limestone per acre applied to all plots, and sulfate of potash and superphosphate applied as usual. Nitrogen omitted from strips 5, 8, and 10.
Non-legume areas seeded with oats; legume areas seeded with mixture of oats and Canada peas. The oats are used to support the peas.

Representative samples of the soil from each plot of the field were taken in the spring of 1931 just before the field was plowed, and again in the fall just after the crop was harvested. Nitrification, cellulose decomposition, and nitrogen fixation were studied in both spring and fall samples.

Nitrification

The technic employed for the study of nitrification was essentially the same as that described by Waksman (6). The soil was dried sufficiently to permit screening through a 20-mesh sieve. The moisture content of the soil was determined in order to obtain desired quantities of the soils in terms of air-dried soil. The nitrate content of the soil as taken from the field was determined. Then three experiments were prepared: The first experiment consisted of 100-gm. quantities of soil with water added to, and maintained at, 60 per cent of saturation. This experiment was designed to study the nitrification of the organic matter present in the soil as taken from the field. The second experiment consisted of 100-gm. quantities of soil with sufficient ammonium sulfate added to supply 50 mgm. of nitrogen in 100 gm. of soil. Water was added to, and maintained at 60 per cent of saturation. The third experiment consisted of 100-gm. quantities of soil to which 1 gm. of dried blood (12 per cent N) per 100 gm. of soil was added. Water was added to, and maintained at, 60 per cent of saturation.

The experiments were set up in fruit jars of 1-pint capacity, and incubated for 4 weeks at 30°C. At the end of the incubation period the nitrate-nitrogen in the different jars was determined.

The procedure for the determination of nitrates was as follows:

The whole content of a jar (100 gm. of soil) was put into a large-mouth bottle with 5 gm. of calcium hydroxide (made by adding distilled water to lumps of calcium oxide until a fine white powder is obtained). After the calcium hydroxide had been mixed with the soil 400 cc. of distilled water was added. The calcium hydroxide was added to remove color from the

filtrate (5). After the water had been added the bottles were tightly stoppered and shaken for 10 minutes in a mechanical shaker. The entire content of a bottle was poured onto a filter paper in a funnel. The filtrate was poured back into the funnel several times until the filtrate was clear. After filtration was completed an aliquot portion of the filtrate was evaporated to dryness and the nitrate content determined by the phenoldisulfonic acid method as employed by Davis (3).

The results of the nitrification studies are shown in tables 1 to 4 inclusive. Variations in the quantities of nitrate are observed in all of the tables but the

TABLE 1

Nitrate in soil as taken from the field

Mgm. NO_3 in 100 gm. soil

STRIP NUMBER	SPRING				FALL			
	L. W.*	N. L. W.	L. E.	N. L. E.	L. W.	N. L. W.	L. E.	N. L. E.
10	7.1	8.8	11.0	6.2	4.4	12.7	8.0	8.8
9	7.1	9.7	6.2	15.1	5.3	9.7	9.7	8.8
8	8.8	9.7	6.2	8.8	6.2	8.8	8.0	8.0
7	10.6	6.2	11.5	9.7	5.3	4.4	8.8	6.2
6	7.1	7.9	7.1	8.8	8.8	8.0	7.1	8.0
5	5.3	7.9	7.1	8.8	7.1	4.4	9.7	7.1

* L. W. = Legume—West; N. L. W. = Non-legume—West; L. E. = Legume—East; N. L. E. = Non-legume—East.

TABLE 2

Nitrification of organic matter of the soil as taken from the field

Mgm. NO_3 in 100 gm. soil

STRIP NUMBER	SPRING				FALL			
	L. W.	N. L. W.	L. E.	N. L. E.	L. W.	N. L. W.	L. E.	N. L. E.
10	19.4	20.3	21.1	19.7	23.9	24.8	20.4	13.3
9	23.8	21.1	18.5	22.9	23.0	17.7	28.4	15.9
8	19.4	20.3	18.5	21.1	25.7	15.9	18.6	21.2
7	16.7	17.4	19.7	19.7	21.2	19.5	19.5	18.6
6	19.4	18.5	19.7	19.7	21.2	16.8	20.4	17.7
5	18.5	18.5	16.7	20.3	21.2	16.8	17.7	17.7

variations do not appear to be related either to the crop or to the soil treatment. There is no indication that the presence of legumes or non-legumes exerted any influence on the nitrifying activity of the soil. Dried blood yielded more nitrate than either the ammonium sulfate or the native organic matter of the soil. There was no apparent effect of season, the nitrification in each of the four experiments indicating that the nitrifying power of soil samples collected in the spring and those collected in the fall were comparable.

Cellulose decomposition

Soils employed for the study of cellulose decomposition were taken from the same samples as those studied for nitrification. They represented samples collected in the spring and samples collected in the fall. The soil was dried sufficiently to permit screening through a 20-mesh sieve. The moisture content of the soil was determined. Then sufficient soil was taken to give 100 gm. of air-dried soil. One gram of finely ground oat straw was thoroughly mixed with each 100-gm. portion of soil. Water was added to 60 per cent of satura-

TABLE 3

Nitrification of ammonium sulfate

Mgm. NO_3 in 100 gm. soil with 50 mgm. N as $(NH_4)_2SO_4$

STRIP NUMBER	SPRING				FALL			
	L. W.	N. L. W.	L. E.	N. L. E.	L. W.	N. L. W.	L. E.	N. L. E.
10	106.3	159.5	141.8	154.1	146.2	150.6	110.7	128.5
9	150.6	132.9	119.6	150.1	146.2	106.3	110.7	128.5
8	141.7	132.9	146.2	154.1	128.5	93.0	101.9	124.0
7	132.9	132.9	106.3	146.2	110.7	119.6	128.5	101.9
6	128.5	115.2	141.8	146.2	101.9	115.2	146.2	88.6
5	110.8	115.2	119.6	146.2	124.0	93.0	101.9	106.3

TABLE 4

Nitrification of dried blood

Mgm. NO_3 in 100 gm. soil with 1 per cent dried blood (12 per cent N)

STRIP NUMBER	SPRING				FALL			
	L. W.	N. L. W.	L. E.	N. L. E.	L. W.	N. L. W.	L. E.	N. L. E.
10	137.3	310.1	296.8	350.0	239.2	248.1	225.9	256.9
9	197.4	252.5	224.0	310.1	256.9	256.9	171.6	168.3
8	251.5	314.5	236.7	332.3	261.4	256.9	256.9	270.2
7	292.4	336.7	281.6	314.5	234.8	292.4	256.9	248.1
6	261.4	354.4	305.6	314.5	252.5	239.2	274.7	261.4
5	287.9	345.3	292.4	310.1	265.8	274.7	243.6	248.1

tion and maintained at that concentration for the duration of the incubation period. The experiments were set up in 500-cc. Erlenmeyer flasks which were closed with rubber stoppers containing inlet and outlet tubes to permit aeration The flasks were incubated at 26°C. until the evolution of carbon dioxide had dropped to a constant level, a period of 12 days. The evolution of carbon dioxide was employed as a measure of the rate of cellulose decomposition. An aeration method described by Waksman and Starkey (8) was employed for determining carbon dioxide. The air from the culture flasks was drawn through 0.25 *N* barium hydroxide, the excess barium hydroxide being titrated back with 0.25 *N* oxalic acid. Phenolphthalein was used as an indicator.

TABLE 5

Carbon dioxide evolution from soil + 1 per cent oat straw

Mgm. CO_2 per 100 gm. of soil, after 12 days

STRIP NUMBER	SPRING				FALL			
	L. W.	N. L. W.	L. E.	N. L. E.	L. W.	N. L. W.	L. E.	N. L. E.
10	371	409	336	322	568	522	467	435
9	347	353	314	358	328	507	533	469
8	394	383	386	363	471	461	508	457
7	395	361	366	339	493	472	482	512
6	396	350	372	402	525	452	546	531
5	397	378	377	362	492	396	512	441

TABLE 6

*Nitrogen fixation**

Mgm. nitrogen increase per 50 cc. Ashby's solution from 1.0 gm., 0.1 gm., and 0.01 gm. quantities of soil

STRIP NUMBER	SOIL	SPRING				FALL			
		L. W.	N. L. W.	L. E.	N. L. E.	L. W.	N. L. W.	L. E.	N. L. E.
	gm.								
10	1.0	+++	+++	+++	+++	+++	+++	+++	+++
	0.1	0	0	+	++	++	+	+	++
	0.01	0	0	0	+	+	0	0	++
9	1.0	+++	+++	+++	+++	+++	+++	+++	+++
	0.1	0	0	++	+++	0	+	+	+++
	0.01	0	0	+	++	0	+	+	+++
8	1.0	+++	++	+++	+++	+++	+++	+++	+++
	0.1	0	+	+++	+++	+	+	++	++
	0.01	0	0	0	0	0	+	+	+
7	1.0	+++	+++	++	+++	+++	+++	+++	+++
	0.1	+	0	+	+	++	+	+	++
	0.01	0	0	0	+	+	+	+	+
6	1.0	++	++	++	+++	++	+++	+++	+++
	0.1	+	+	+	+	++	++	+	+
	0.01	0	0	0	0	++	+	+	+
5	1.0	++	+++	++	+++	+++	+++	+++	+++
	0.1	0	0	+	+++	0	+	+	++
	0.01	0	0	0	+	0	+	+	+

* +++ = Over 2 mgm. nitrogen per culture.
++ = 1 to 2 mgm. nitrogen per culture.
+ = Less than 1 mgm. nitrogen per culture.
0 = No nitrogen increase.

The results of the cellulose decomposition studies are shown in table 5. There is no indication that the process was influenced by the presence of legume or non-legume crops. In that respect the studies agree with the nitrification studies. Carbon dioxide evolution was greater in the soil samples collected in the fall than in those collected in the spring, indicating that the decomposition of cellulose was more active in the fall. This increase of activity appeared to be general over the entire experimental field.

Nitrogen fixation

In order to compare the functions of nitrification and cellulose decomposition with that of nitrogen fixation in a given season, nitrogen fixation studies were made with the soil samples collected in the spring and those collected in the fall. The procedure was the same as that used in the previously published (2) study of this series. The results were in agreement with those previously published, in that the legumes or non-legumes did not appear to influence nitrogen fixation by the soil microörganisms in cultures. Since the results obtained in the present study were comparable with those published earlier (2) no tables are included in this paper.

A study of the distribution of nitrogen-fixing bacteria in the soil of the various plots was made by a dilution method. Blake culture bottles were prepared, each containing 50 cc. of Ashby's solution (1). One gram, 0.1 gm. and 0.01 gm. respectively from each soil sample were inoculated into culture bottles. After two weeks of incubation the nitrogen increase was determined by the Kjeldahl method. The results are shown in table 6. It will be observed that the east half of the field, the legume-east and non-legume-east strips, showed more samples of soil giving some nitrogen increase in the 0.01 gm. inoculations than was obtained with soils from the west half of the field. The only reason that can be given is that the field slopes downward from west to east with the result that the moisture retention was probably greater in the east half of the field. The results do not warrant any assumption that the presence of legumes or non-legumes had any influence on the amount of nitrogen fixed.

SUMMARY

Nitrification of ammonium sulfate, dried blood, and the native organic matter by the soil from different plots of an experimental field did not appear to be influenced by the presence of legume or non-legume crops in the field. The results obtained from soil samples collected in the spring and in the fall did not differ significantly

Cellulose (oat straw) decomposition in soil from the different plots of the field, as indicated by the evolution of carbon dioxide, did not appear to be influenced by the presence of legume or non-legume crops in the field. The evolution of carbon dioxide was greater in the samples collected in the fall than in samples collected in the spring, indicating that cellulose decomposition was more active in the fall.

Nitrogen fixation studies with the soil from the different plots of the field gave results comparable with those previously published (2). Nitrogen fixation did not appear to be influenced by the presence of legume or non-legume crops in the field.

Nitrification, cellulose decomposition, and nitrogen fixation studied gave results which were in agreement, in that there was apparently no influence exerted by the presence of legume or non-legume crops in the field.

The results of chemical analyses of crops taken from the field, and of the soil from the field, have been published by Morse (4).

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